

Supplementary material for the article:

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## Supplementary Material

### ***4-Aminoquinoline-based compounds as antileishmanial agents that inhibit the energy metabolism of Leishmania***

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**Table S1.** Lethal and Teratogenic Effects Observed in Zebrafish (*Danio rerio*) Embryos at Different Hours post Fertilization (hpf)

Category	Developmental endpoints	Exposure time (hpf)				
		24	48	72	96	120
<b>Lethal effect</b>	Coagulated eggs	●	●	●	●	●
	Lack of the heart beating	●	●	●	●	●
<b>Teratogenic effect</b>	Malformation of head	●	●	●	●	●
	Malformation of eyes	●	●	●	●	●
	Malformation of sacculi/otoliths	●	●	●	●	●
	Malformation of chorda	●	●	●	●	●
	Malformation of tail	●	●	●	●	●
	Scoliosis	●	●	●	●	●
	Yolk edema	●	●	●	●	●
	Yolk deformation	●	●	●	●	●
	Growth retardation			●	●	●
	Hatching				●	●
<b>Cardiotoxicity</b>	Pericardial edema			●	●	●
	Heart morphology				●	●
	Heart beating rate (beat/min)					●

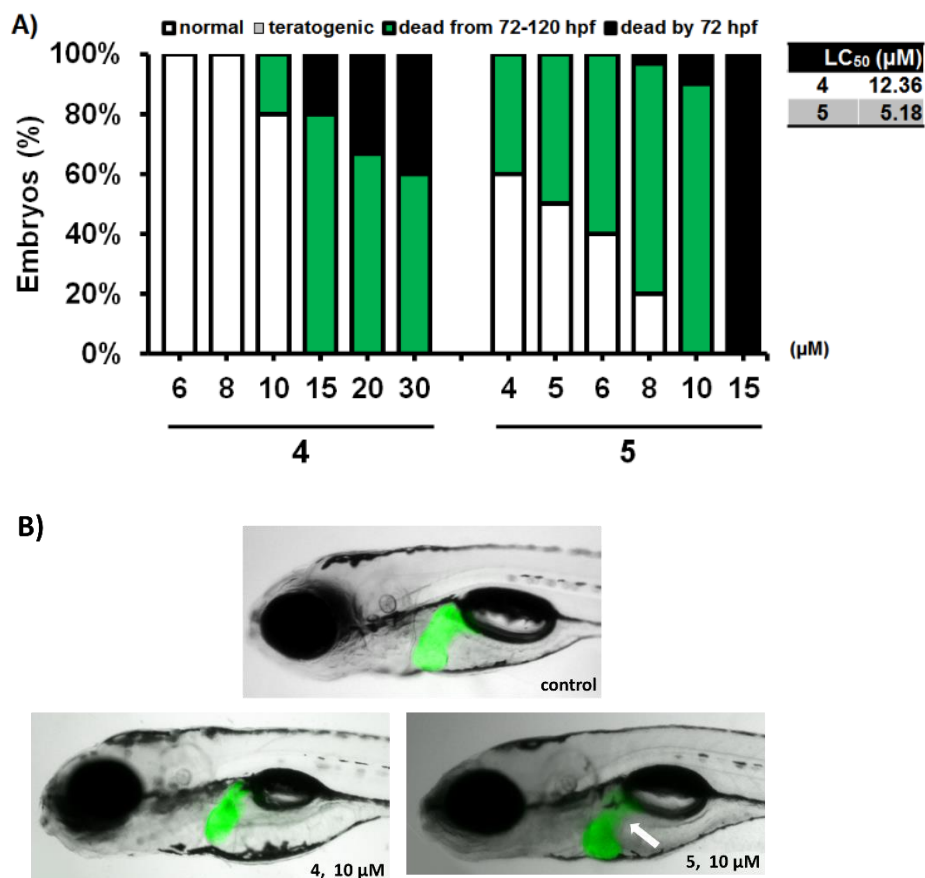
<sup>a</sup>No clear organs structure is recognized.

<sup>b</sup>Malformation of eyes was recorded for the retardation in eye development and abnormality in shape and size.

<sup>c</sup>Presence of none, one or more than two otoliths per sacculus, as well as reduction and enlargement of otoliths and/or sacculi (otic vesicles).

<sup>d</sup>Tail malformation was recorded when the tail was bent, twisted or shorter than to control embryos as assessed by optical comparison.

<sup>e</sup>Growth retardation was recorded by comparing with the control embryos in a body length (after hatching, at and onwards 72 hpf) using by optical comparison using an inverted microscope (CKX41; Olympus, Tokyo, Japan).

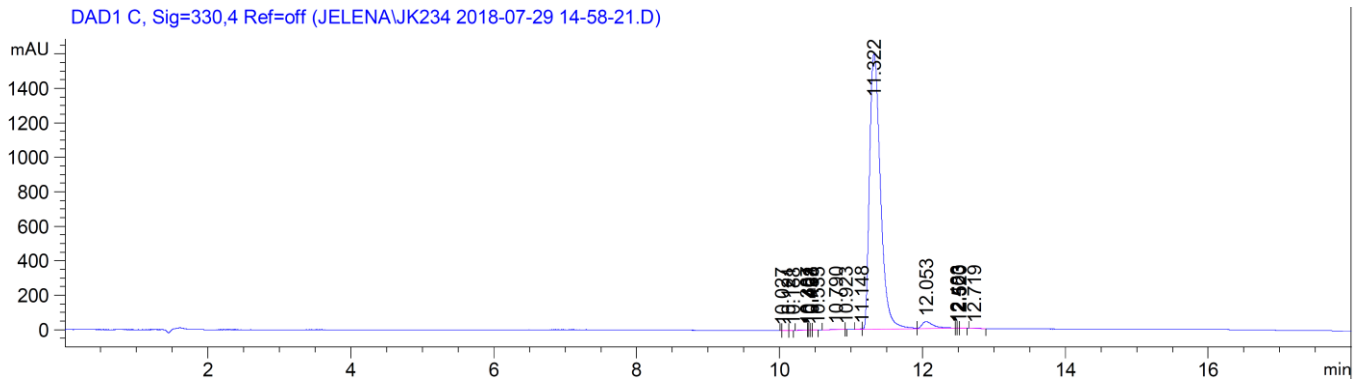


**Figure S1.** Toxicity evaluation in the zebrafish model. The treated zebrafish embryos were followed for the survival and teratogenicity (A) as well as hepatotoxicity (B). Hepatotoxicity evaluation was performed in the transgenic Tg(fabp10:EGFP) zebrafish embryos with fluorescently labelled liver (green color), that were exposed to the tested compounds at 72 hpf onwards (the stage when the liver become vascularized and performed metabolic transformation of the absorbed compounds). At 120 hpf stage, the embryos upon 10  $\mu\text{M}$  of **4** had normally developed liver as those in the control (DMSO-treated) group, while the embryos upon 10  $\mu\text{M}$  of **5** showed darker liver with less green fluorescence (arrow) as clear signs of the liver necrosis.

## **HPLC analyses for purity**

## Compound: 2

### Method A

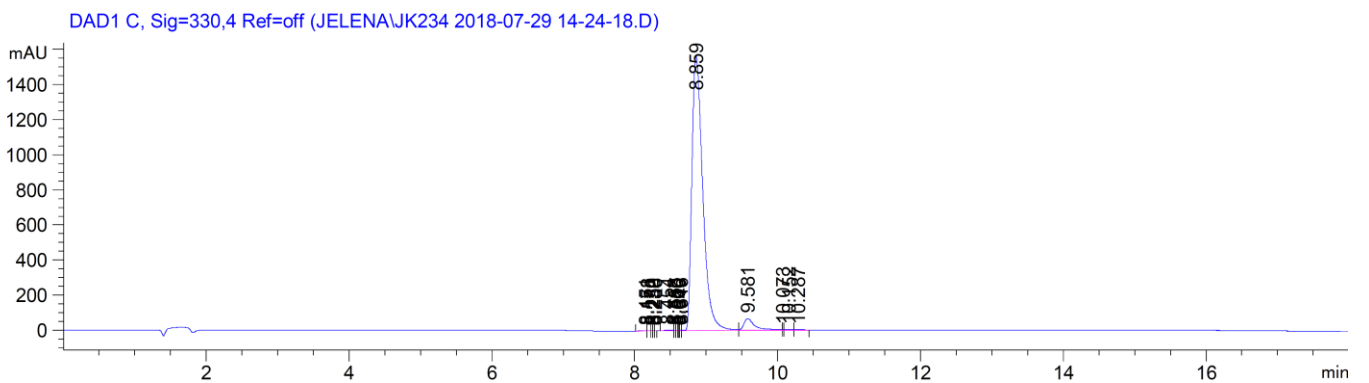


Signal 2: DAD1 C, Sig=330,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	10.027	BB	0.0155	1.45981e-1	1.21776e-1	8.400e-4
2	10.121	BV	0.0455	4.55632e-1	1.22114e-1	2.622e-3
3	10.188	VB	0.0332	2.92213e-1	1.09100e-1	1.681e-3
4	10.387	BV	0.1214	3.61849	3.53683e-1	0.0208
5	10.401	VV	0.0104	1.99608e-1	2.76385e-1	1.149e-3
6	10.428	VB	0.0193	2.15344e-1	1.40871e-1	1.239e-3
7	10.453	BB	0.0164	1.11127e-1	9.02968e-2	6.394e-4
8	10.535	BB	0.0360	3.52593e-1	1.19400e-1	2.029e-3
9	10.790	BV	0.0972	23.21344	2.85920	0.1336
10	10.923	VB	0.0174	3.03519e-1	2.64300e-1	1.746e-3
11	11.148	BV	0.0431	6.16876	1.77288	0.0355
12	11.322	VV	0.1494	1.68941e4	1604.02917	97.2084
13	12.053	VB	0.1420	430.61771	39.55185	2.4778
14	12.483	BV	0.0126	4.02082e-1	4.22963e-1	2.314e-3
15	12.503	VV	0.0183	1.06655	7.39961e-1	6.137e-3
16	12.520	VB	0.0452	3.14331	8.58255e-1	0.0181
17	12.719	BV	0.0776	14.85247	2.29311	0.0855

Totals : 1.73793e4 1654.12532

### Method B



Signal 2: DAD1 C, Sig=330,4 Ref=off

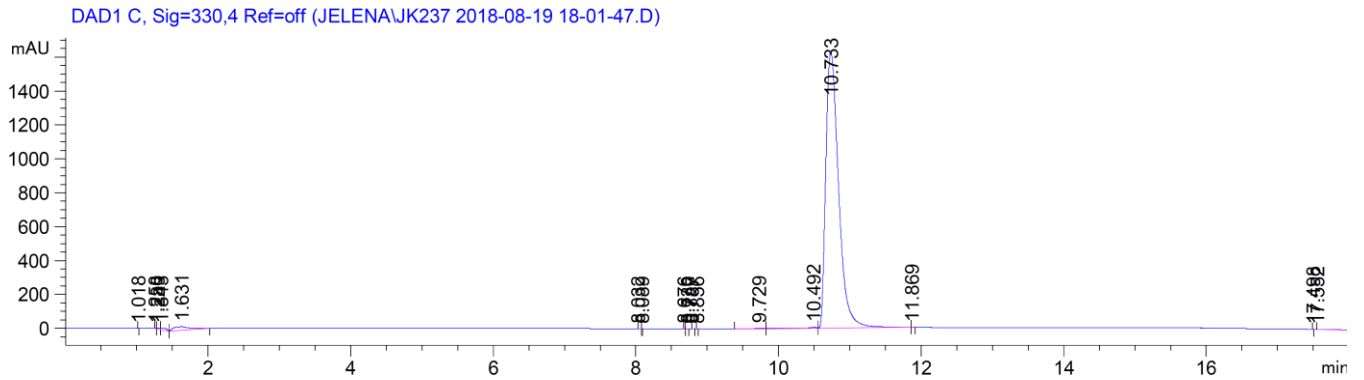
Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	8.151	BV	0.0780	9.76215	1.49910	0.0567
2	8.178	VV	0.0283	2.59204	1.14956	0.0151
3	8.232	VB	0.0143	4.24840e-1	4.18961e-1	2.468e-3
4	8.259	BB	0.0155	1.22136e-1	1.05606e-1	7.095e-4
5	8.286	BB	0.0127	1.00358e-1	1.09124e-1	5.830e-4
6	8.454	BV	0.0717	12.40781	2.06313	0.0721
7	8.553	VB	0.0140	2.76956e-1	2.80953e-1	1.609e-3
8	8.580	BB	0.0126	1.01489e-1	1.06899e-1	5.896e-4
9	8.606	BV	7.20e-3	5.45764e-2	1.22509e-1	3.171e-4
10	8.619	VB	8.38e-3	4.10111e-2	7.49530e-2	2.383e-4
11	8.646	BB	0.0135	1.28014e-1	1.24801e-1	7.437e-4
12	8.859	BV	0.1520	1.64482e4	1560.66370	95.5553
13	9.581	VV	0.1464	693.53467	65.56276	4.0291
14	10.073	VV	0.0184	2.98274	2.05775	0.0173
15	10.152	VV	0.0800	18.38502	2.75028	0.1068
16	10.287	VB	0.0806	24.16438	3.60783	0.1404

Totals : 1.72133e4 1640.69791



### Compound: 3

### Method A

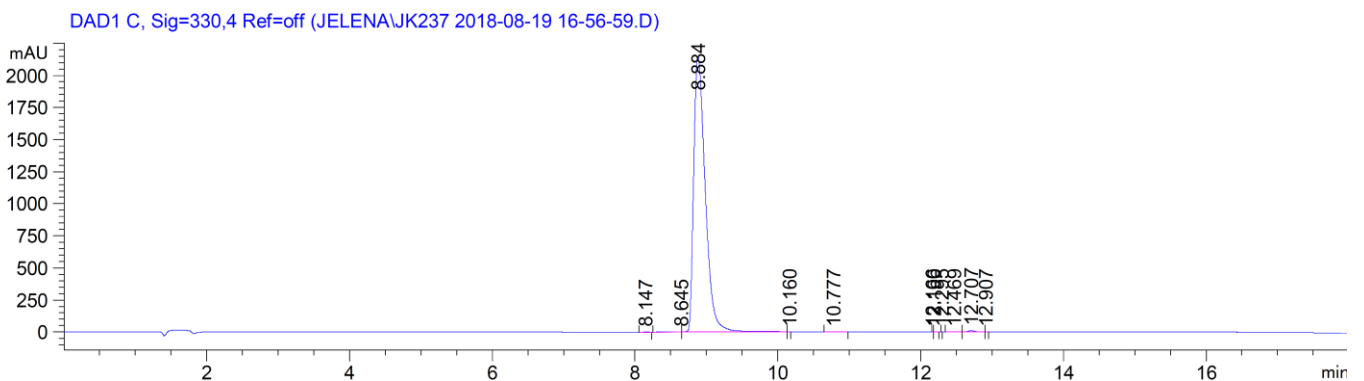


Signal 2: DAD1 C, Sig=330,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.018	BB	0.0103	6.80447e-2	9.03013e-2	3.281e-4
2	1.258	BV	0.0372	2.03269	6.55364e-1	9.802e-3
3	1.289	VV	0.0551	13.97275	3.01355	0.0674
4	1.343	VB	0.0928	54.94912	7.13524	0.2650
5	1.631	BB	0.2052	344.16910	20.61899	1.6596
6	8.032	BB	0.0265	1.38903e-1	6.60560e-2	6.698e-4
7	8.089	BB	8.56e-3	4.18853e-2	6.91993e-2	2.020e-4
8	8.676	BB	0.0118	6.38190e-2	7.19585e-2	3.077e-4
9	8.730	BB	0.0155	9.81092e-2	8.16774e-2	4.731e-4
10	8.787	BB	0.0245	1.30977e-1	6.65189e-2	6.316e-4
11	8.856	BB	0.0140	5.44310e-2	5.51887e-2	2.625e-4
12	9.729	BV	0.1059	18.21764	2.03598	0.0878
13	10.492	VV	0.1229	53.38946	5.19486	0.2574
14	10.733	VV	0.1827	2.02379e4	1631.32861	97.5874
15	11.869	VB	0.0237	7.46707e-1	3.92920e-1	3.601e-3
16	17.498	BB	6.99e-3	2.65479e-2	6.20976e-2	1.280e-4
17	17.552	BBA	1.1846	12.22327	1.20737e-1	0.0589

Totals : 2.07383e4 1671.05926

### Method B



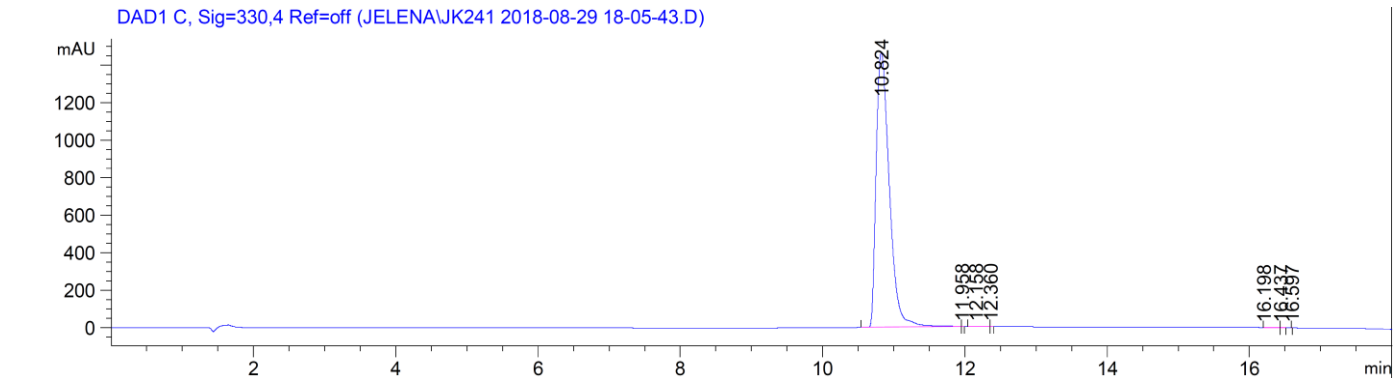
Signal 2: DAD1 C, Sig=330,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	8.147	BB	0.0618	12.70354	2.52128	0.0524
2	8.645	BV	0.1545	36.76767	2.81226	0.1517
3	8.884	VV	0.1380	2.40994e4	2146.44434	99.4338
4	10.160	VB	0.0343	3.61656e-1	1.30772e-1	1.492e-3
5	10.777	BB	0.1148	7.77478	8.07792e-1	0.0321
6	12.166	BV	0.0123	1.11128e-1	1.25482e-1	4.585e-4
7	12.190	VB	0.0344	4.45103e-1	1.67969e-1	1.836e-3
8	12.295	BB	8.83e-3	4.39644e-2	6.99601e-2	1.814e-4
9	12.469	BV	0.0722	16.14988	2.68476	0.0666
10	12.707	VV	0.0903	62.56902	9.62711	0.2582
11	12.907	VB	0.0237	2.97840e-1	1.67982e-1	1.229e-3

Totals : 2.42366e4 2165.55971

# Compound: 4

## Method A

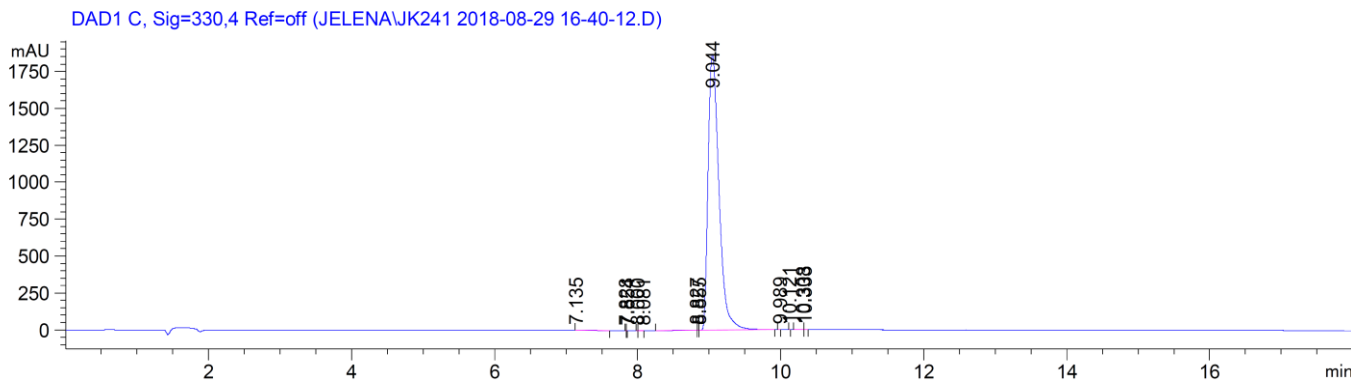


Signal 2: DAD1 C, Sig=330,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	10.824	BV	0.1918	1.88262e4	1466.37073	99.9542
2	11.958	VB	0.0166	4.22714e-1	3.38008e-1	2.244e-3
3	12.158	BB	0.1050	6.82548	7.69739e-1	0.0362
4	12.360	BB	0.0193	1.48503e-1	1.00303e-1	7.884e-4
5	16.198	BB	0.1097	9.53674e-1	1.03330e-1	5.063e-3
6	16.437	BB	0.0317	2.42020e-1	9.68210e-2	1.285e-3
7	16.597	BB	6.66e-3	3.57628e-2	8.94990e-2	1.899e-4

Totals : 1.88348e4 1467.86843

## Method B



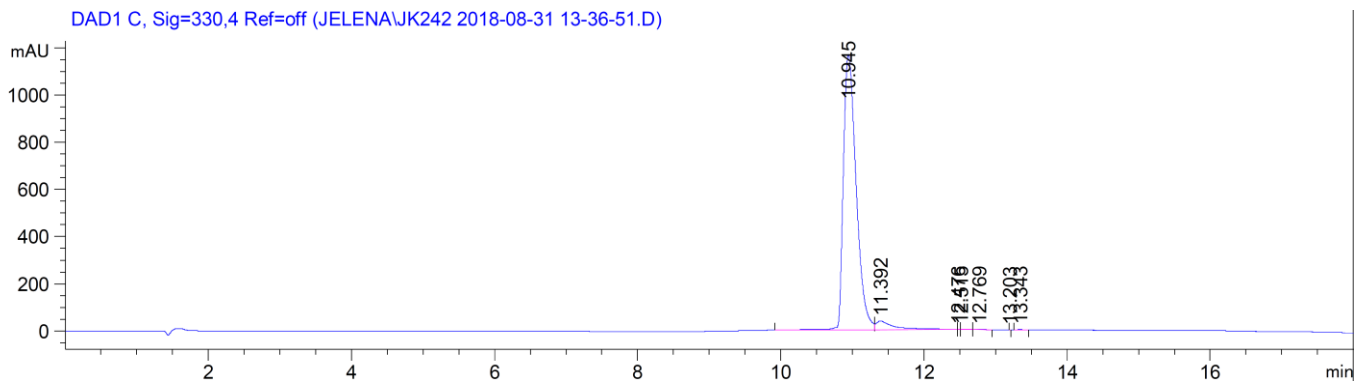
Signal 2: DAD1 C, Sig=330,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	7.135	BB	0.9501	11.18469	1.37630e-1	0.0566
2	7.828	BB	6.72e-3	3.39404e-2	8.39444e-2	1.717e-4
3	7.854	BB	7.75e-3	4.22052e-2	7.87832e-2	2.135e-4
4	8.000	BB	0.0139	7.44843e-2	7.63582e-2	3.769e-4
5	8.081	BB	0.0318	2.13756e-1	8.37574e-2	1.082e-3
6	8.827	BV	0.2494	31.61536	1.49071	0.1600
7	8.855	VV	0.0205	2.37039	1.45253	0.0120
8	9.044	VB	0.1428	1.97159e4	1866.05652	99.7533
9	9.989	BB	0.0199	1.28725e-1	8.37171e-2	6.513e-4
10	10.121	BB	0.0105	4.93526e-2	6.78113e-2	2.497e-4
11	10.308	BV	0.0520	2.35021	5.65105e-1	0.0119
12	10.333	VV	0.0225	6.97055e-1	3.96361e-1	3.527e-3

Totals : 1.97647e4 1870.57322

# Compound: 5

## Method A

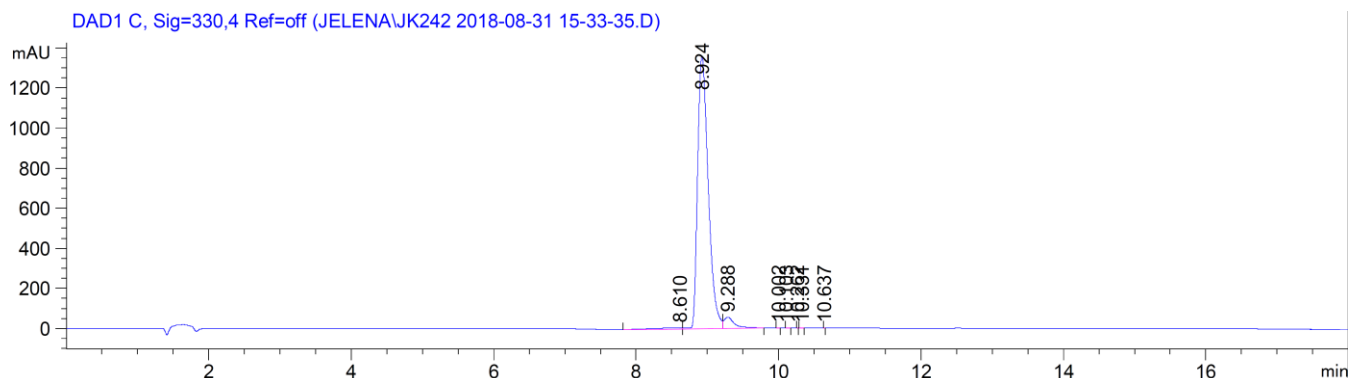


Signal 2: DAD1 C, Sig=330,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	10.945	BV	0.1914	1.44517e4	1167.58459	95.2895
2	11.392	VV	0.2361	692.07031	37.28458	4.5633
3	12.476	VV	0.0261	1.72023	8.15765e-1	0.0113
4	12.515	VB	0.0619	3.71449	7.29784e-1	0.0245
5	12.769	BB	0.0752	7.34643	1.16341	0.0484
6	13.203	BB	9.45e-3	3.52383e-2	5.91132e-2	2.323e-4
7	13.343	BB	0.0681	9.51056	1.66680	0.0627

Totals : 1.51661e4 1209.30404

## Method B



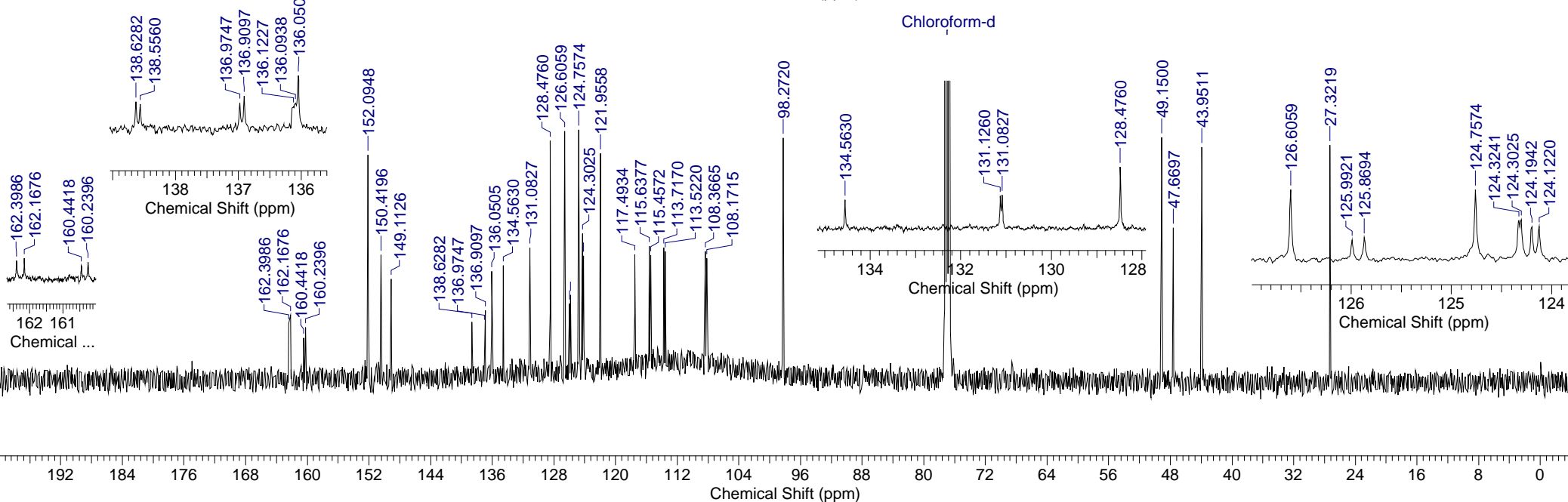
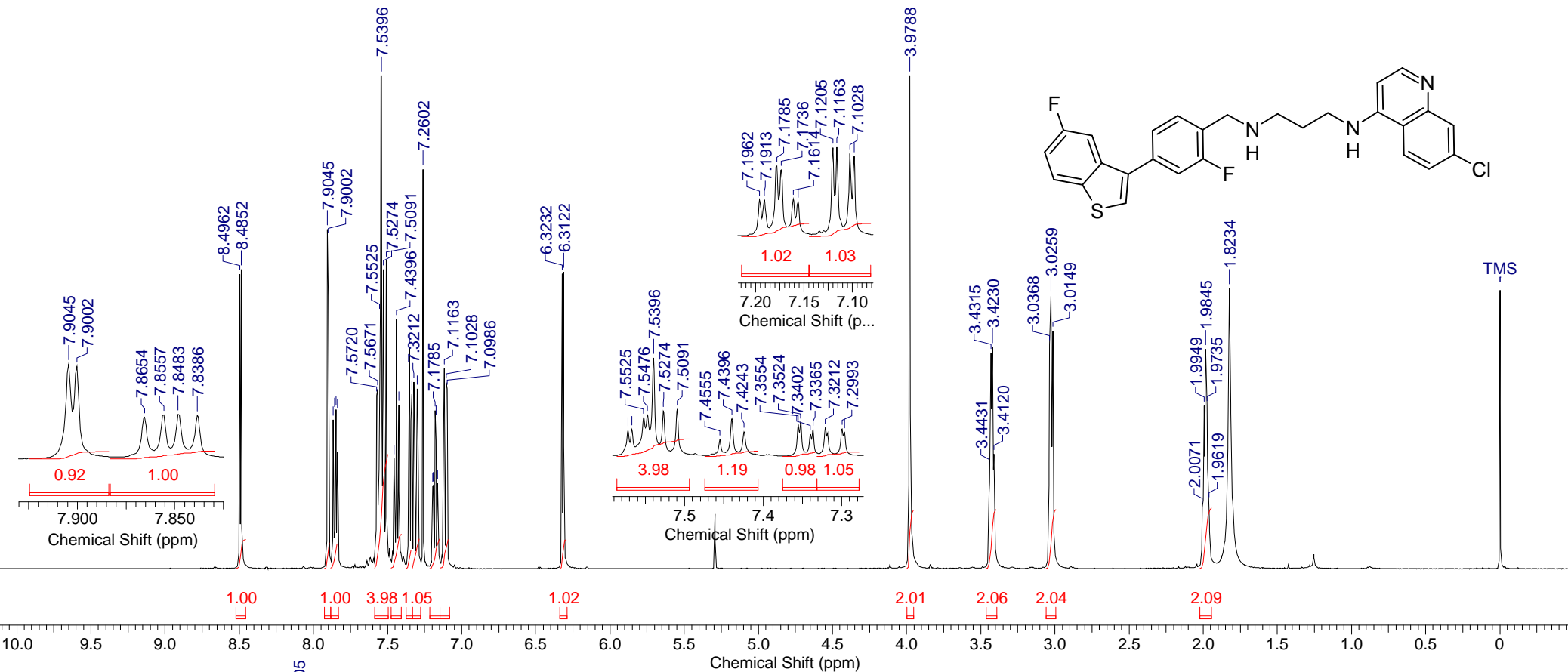
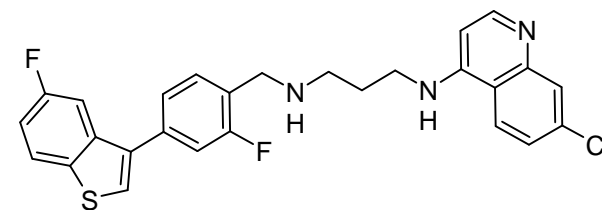
Signal 2: DAD1 C, Sig=330,4 Ref=off

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	8.610	BV	0.3284	139.80365	4.99738	0.9469
2	8.924	VV	0.1614	1.40790e4	1358.50659	95.3557
3	9.288	VB	0.1382	545.21613	55.21946	3.6927
4	10.002	BB	0.0228	1.55115e-1	8.68322e-2	1.051e-3
5	10.103	BB	0.0397	2.36833e-1	7.23824e-2	1.604e-3
6	10.262	BB	6.86e-3	2.47573e-2	5.39274e-2	1.677e-4
7	10.331	BB	0.0227	2.39671e-1	1.31896e-1	1.623e-3
8	10.637	BB	0.0110	5.02586e-2	6.17247e-2	3.404e-4

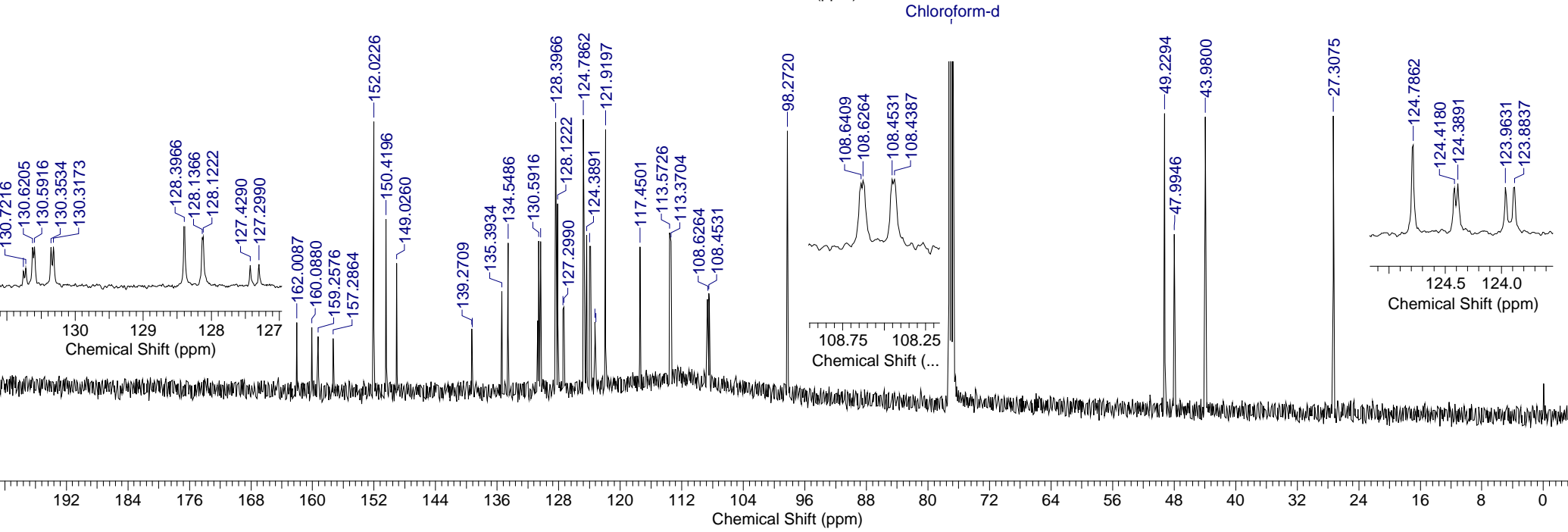
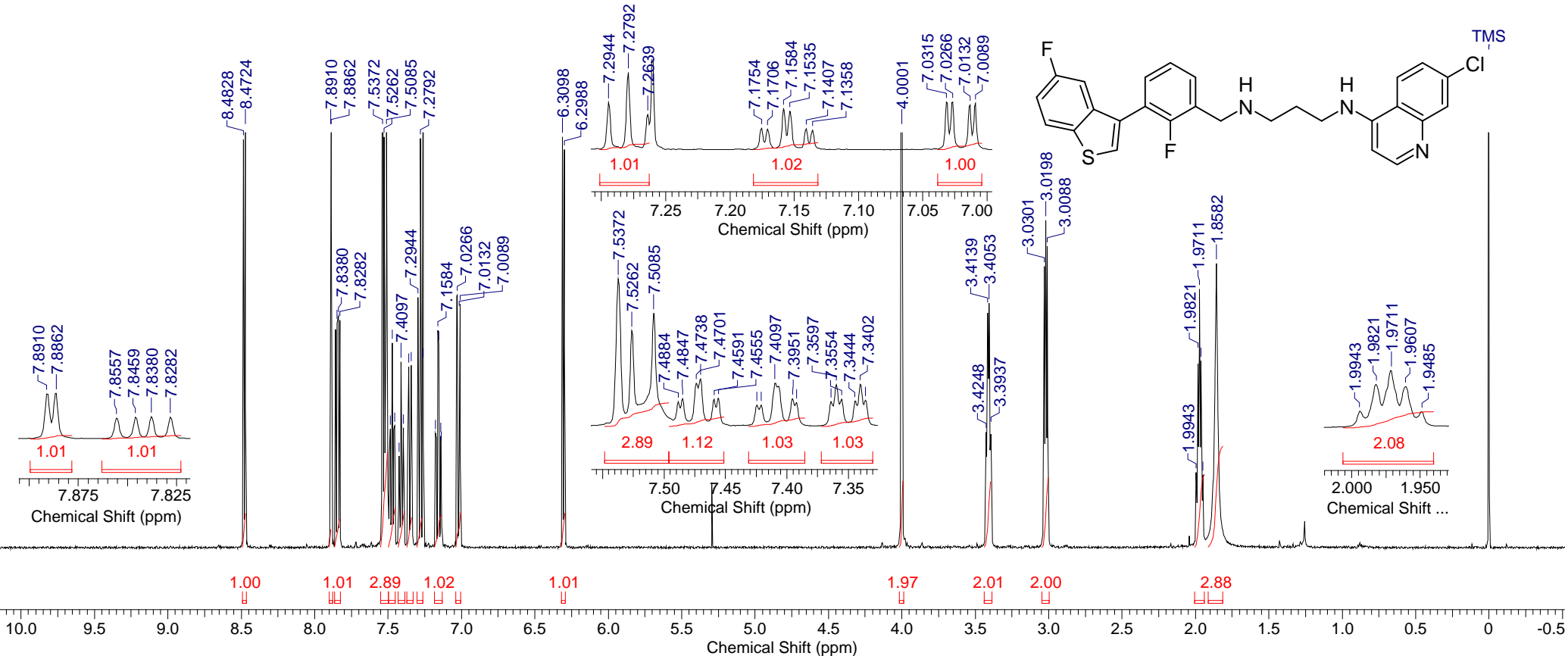
Totals : 1.47648e4 1419.13019

## **NMR spectra of synthesized compounds**

**N-(7-chloroquinolin-4-yl)-N'-[2-fluoro-4-(5-fluoro-1-benzothien-3-yl)benzyl]propane-1,3-diamine (2)**

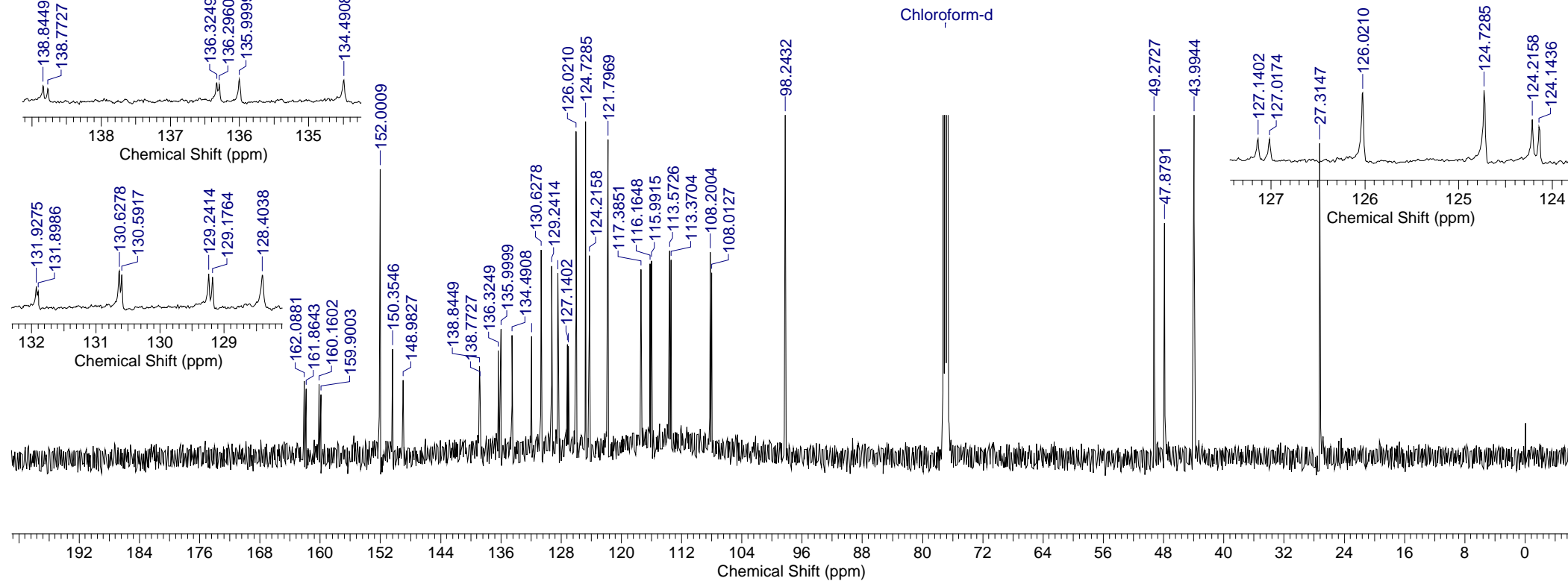
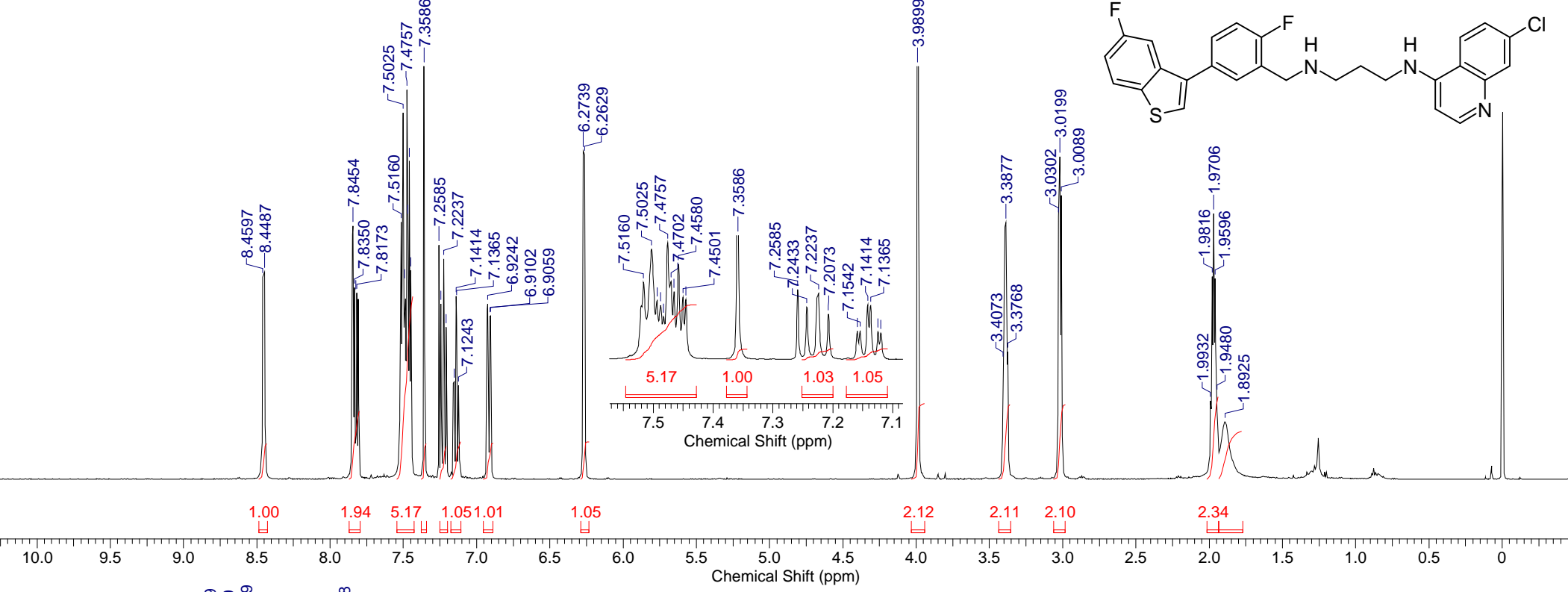
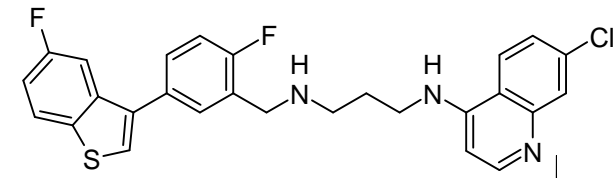


***N*-(7-chloroquinolin-4-yl)-*N'*-[2-fluoro-3-(5-fluoro-1-benzothien-3-yl)benzyl]propane-1,3-diamine (3)**





***N*-(7-chloroquinolin-4-yl)- *N'*-[2-fluoro-5-(5-fluoro-1-benzothien-3-yl)benzyl]propane-1,3-diamine (4)**



**N-(7-chloroquinolin-4-yl)-N'-{1-[4-(5-fluoro-1-benzothien-3-yl)phenyl]ethyl}propane-1,3-diamine (5)**

