

Supporting Information for

**New bioluminescent coelenterazine derivatives with various C-6
substitutions**

Tianyu Jiang^a, Xingye Yang^a, Yubin Zhou^b, Ilia Yampolsky,^{c,d} Lupei Du^a and Minyong Li^{a,*}

^a Department of Medicinal Chemistry, Key Laboratory of Chemical Biology (MOE), School of Pharmaceutical Sciences, Shandong University, Jinan, Shandong 250012, China. Tel./fax: +86-531-8838-2076; e-mail: mli@sdu.edu.cn

^b Center for Translational Cancer Research, Institute of Biosciences & Technology, Texas A&M University Health Science Center, Houston, TX 77030, USA

^c Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry of the Russian Academy of Sciences, Miklukho-Maklaya, 16/10, Moscow 117997, Russia

^d Pirogov Russian National Research Medical University, Ostrovitianov 1, Moscow 117997, Russia

Content

Bioluminescence properties.....	S1
Toxicity of novel Coelenterazine derivatives.....	S2
Other properties	S2
HPLC spectra, NMR spectra and HR-MS spectra	S3

Bioluminescence properties

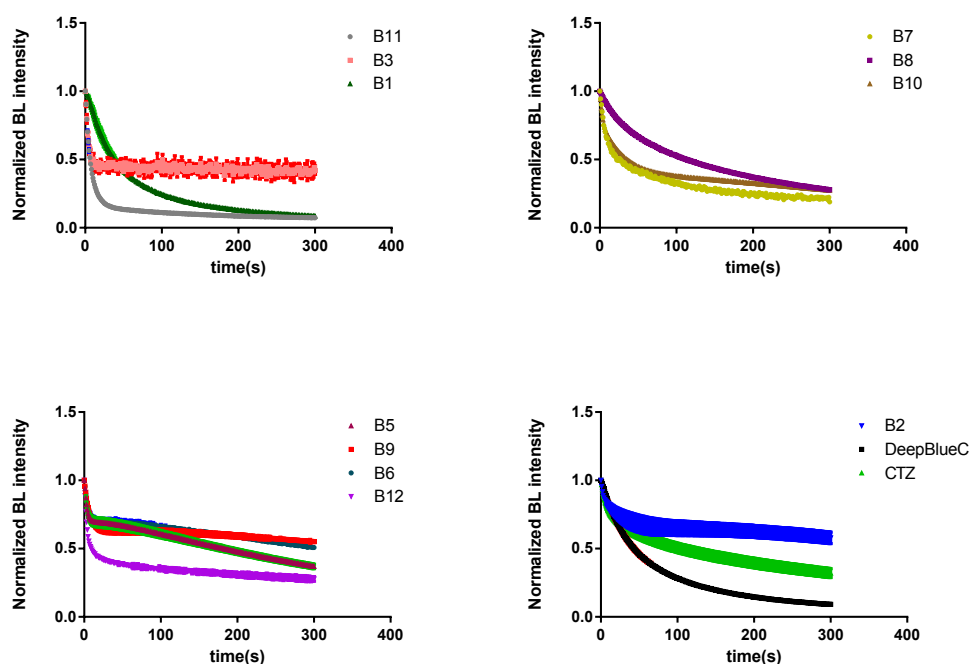


Figure S1. Kinetic profile of bioluminescence of coelenterazine derivatives with *Renilla* luciferase.

Table S1. Bioluminescence properties of all compounds with *Gaussia* luciferase.

Compounds	Emission (nm)	Half life ^a (s)	K_m^b (μ M)	V_{max}^b	Intensity (5 μ M)%
DeepBlueC™	508	27.1	22.97 ± 2.93	$(1.24 \pm 0.09) \times 10^4$	100
CTZ	503	34.5	16.57 ± 1.47	$(1.26 \pm 0.04) \times 10^6$	80401
B1	507	52.6	2.16 ± 0.25	$(1.75 \pm 0.06) \times 10^3$	57
B2	504	11.6	4.38 ± 0.56	$(4.81 \pm 0.19) \times 10^5$	3876
B3	511	28.9	5.76 ± 0.78	$(8.77 \pm 0.47) \times 10^3$	218
B4	N.A.	N.A.	N.A.	N.A.	N.A.
B5	510	33.7	5.32 ± 0.38	$(1.04 \pm 0.03) \times 10^4$	234
B6	490	71.2	1.34 ± 0.11	$(3.33 \pm 0.07) \times 10^2$	12
B7	509	43.9	0.55 ± 0.057	$(1.37 \pm 0.03) \times 10^3$	62
B8	477	12.3	8.20 ± 0.42	$(1.09 \pm 0.02) \times 10^4$	201
B9	499	36.7	2.16 ± 0.32	$(1.16 \pm 0.05) \times 10^3$	33
B10	510	25.9	9.75 ± 1.05	$(1.84 \pm 0.06) 10^4$	121
B11	503	30.7	29.96 ± 4.96	$(7.47 \pm 0.78) \times 10^3$	58
B12	508	51.36	4.81 ± 0.37	$(3.41 \pm 0.07) \times 10^3$	92

^a BL half-life was calculated using GraphPad Prism software and taken advantage of the values when compounds were 25 μ M.

^b Michaelis constant K_m and maximum rate V_{max} were estimated with the Michaelis–Menten kinetics equation using GraphPad Prism software. The values are shown by means $\pm$ SD of three independent assays performed in triplicate.

Toxicity of novel Coelenterazine derivatives

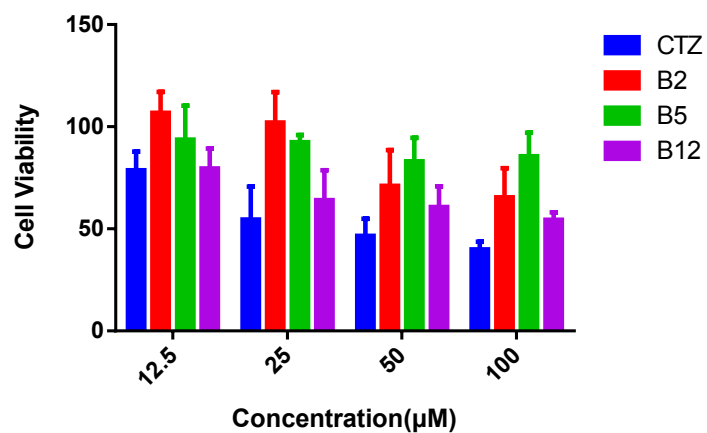


Figure S2. The cell viability of ES-2 cells expressing *Renilla luciferase* (Rluc) incubated with B2, B5, B12 and coelenterazine for 2 hours.

Other properties

Table S2. Chemiluminescence maximum wavelength and fluorescence properties.

Compounds	Chemluminescence Emission (nm)	Fluorescence Excitation (nm)	Fluorescence Emission (nm)
DeepBlueC™	486	455	535
B1	489	415	535
B2	495	415	530
B3	481	415	530
B4	502	335	400
B5	499	355	475
B6	500	345	430
B7	564	410	540
B8	487	310	460
B9	496	345	430
B10	490	415	545
B11	500	335	427
B12	494	350	430

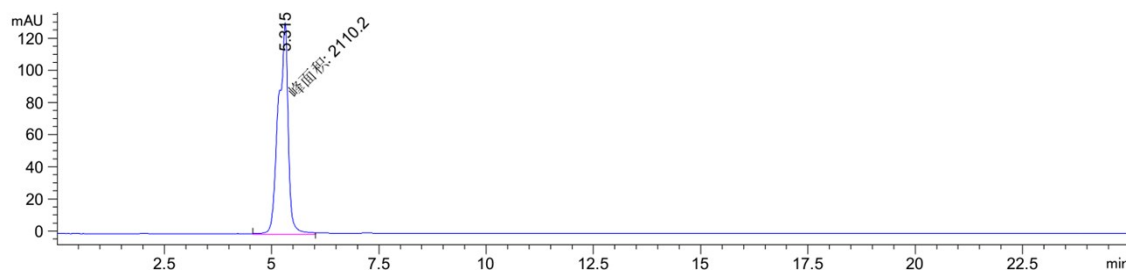
Table S3. ClogP values of compounds (calculated by ChemDraw software)

	DeepBlueC™	Coelenterazine	B2	B5	B9	B12
CLogP	5.52	4.42	4.64	5.41	4.94	5.61

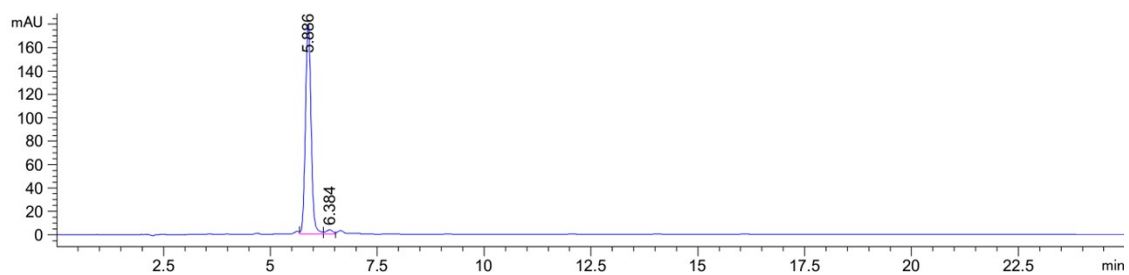
HPLC spectra, NMR spectra and HR-MS spectra

HPLC spectra

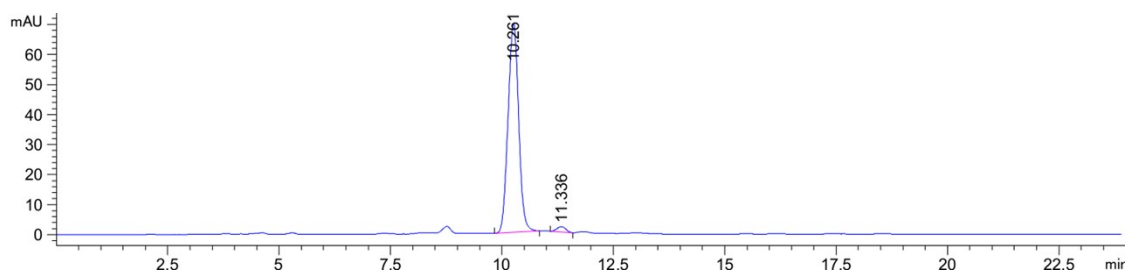
(B1)



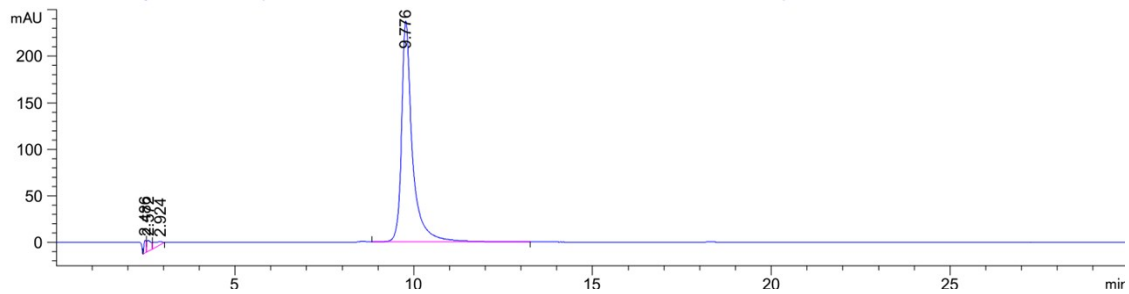
(B2)



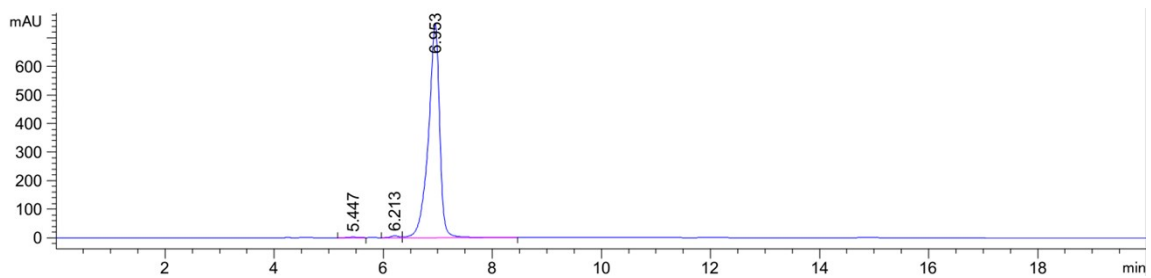
(B3)



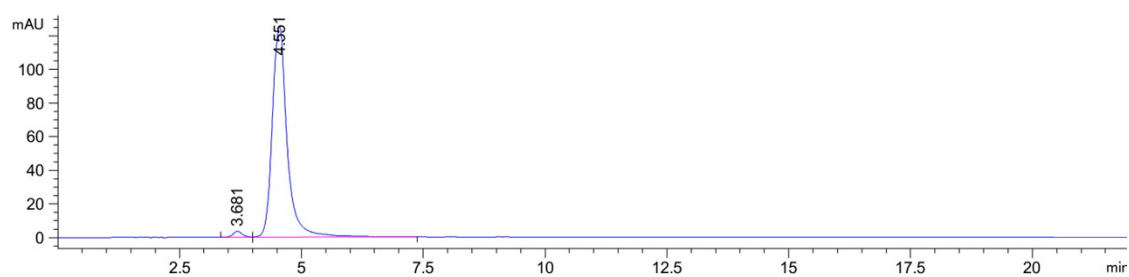
(B4)



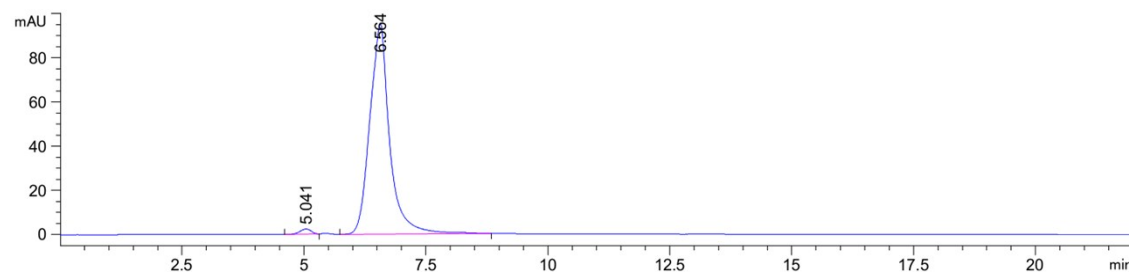
(B5)



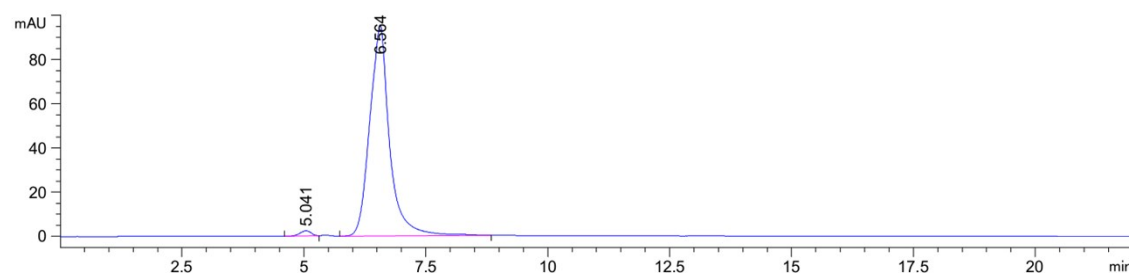
(B6)



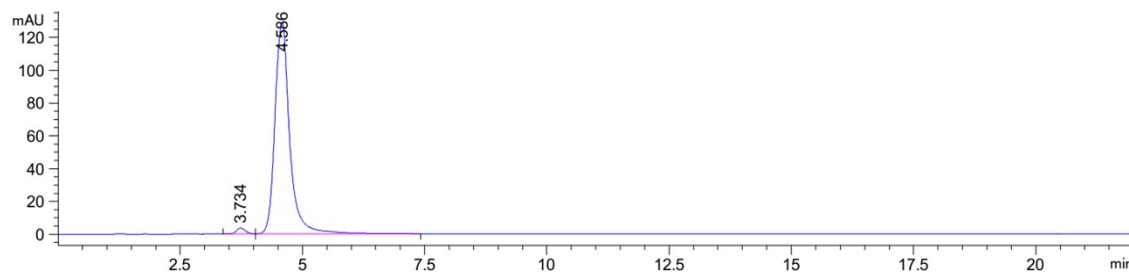
(B7)



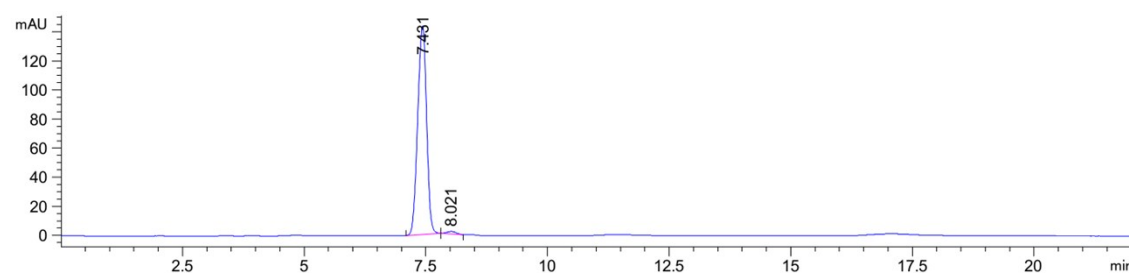
(B8)



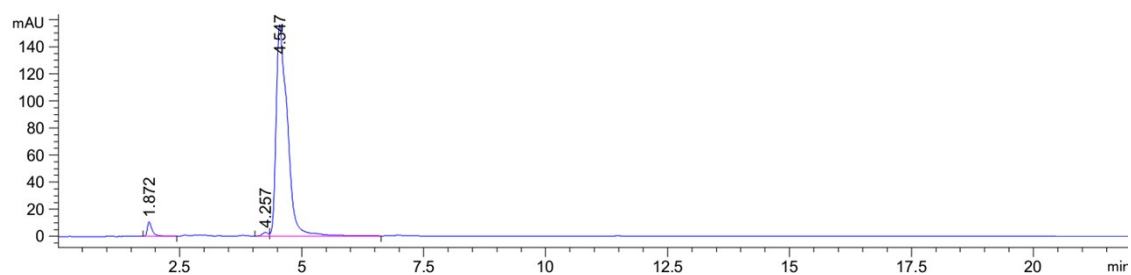
(B9)



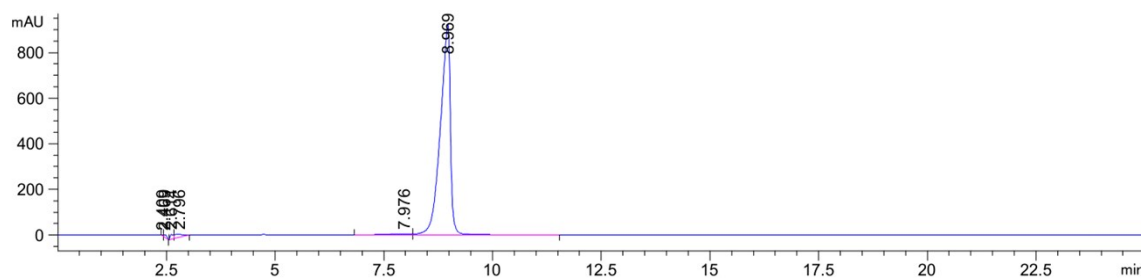
(B10)



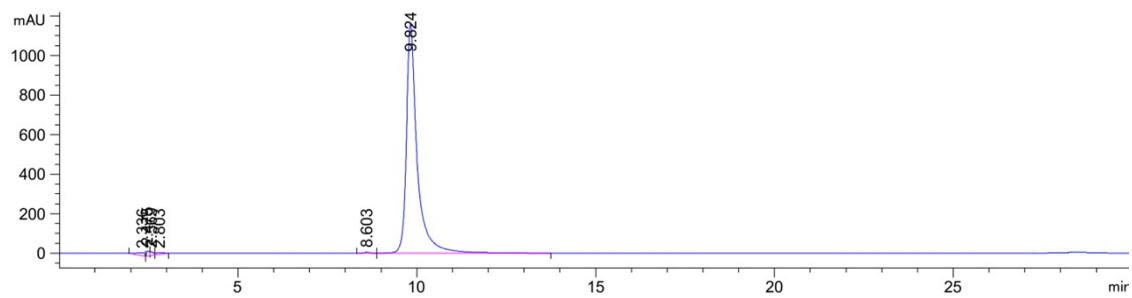
(B11)



(B12)

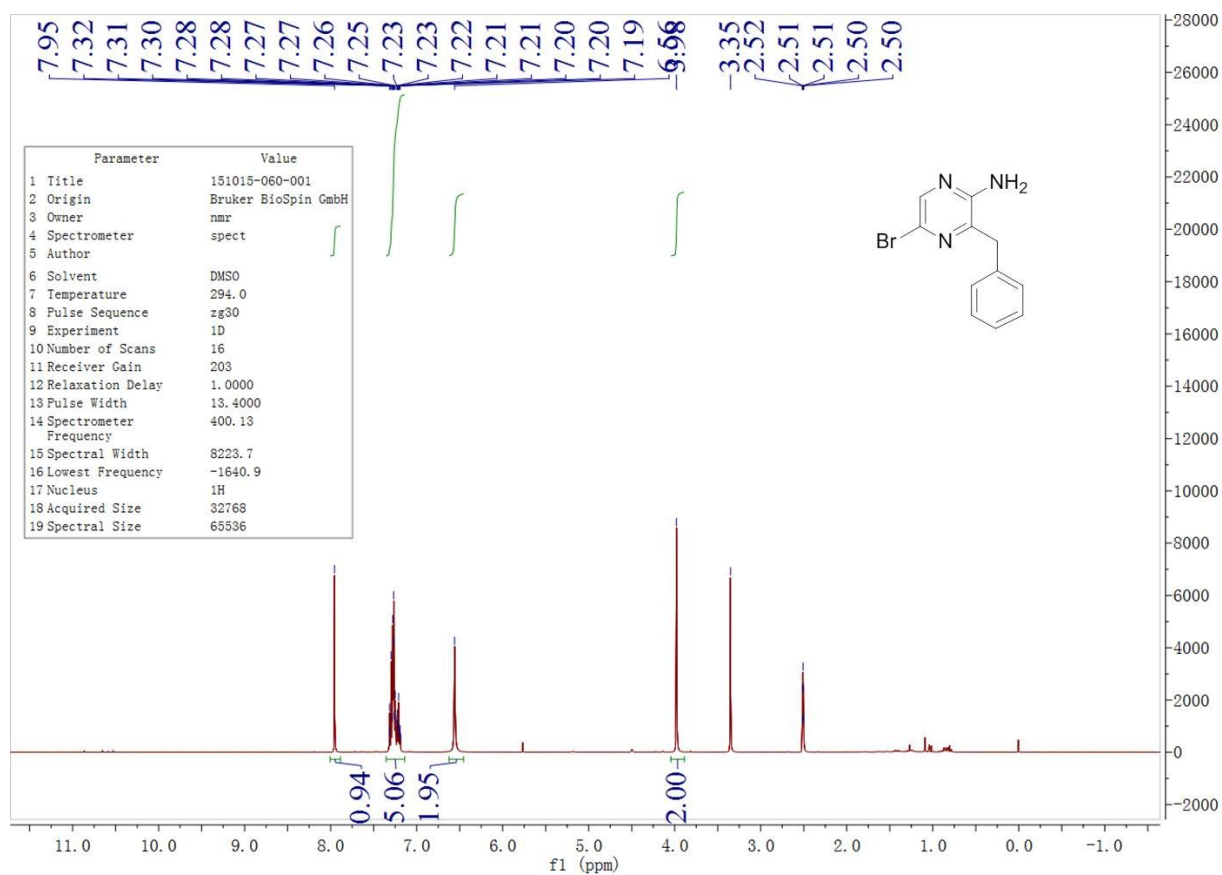


(B13 DeepBlueC™)

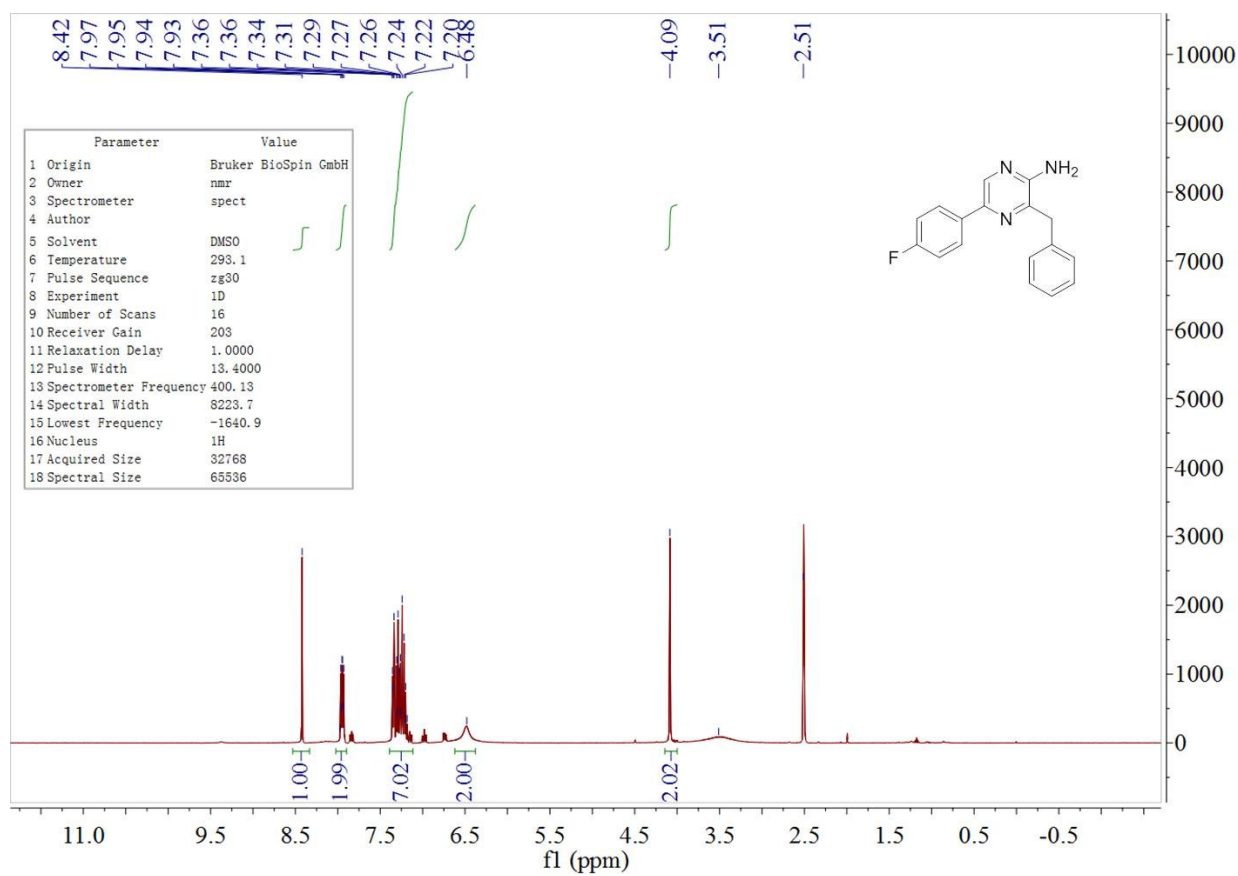


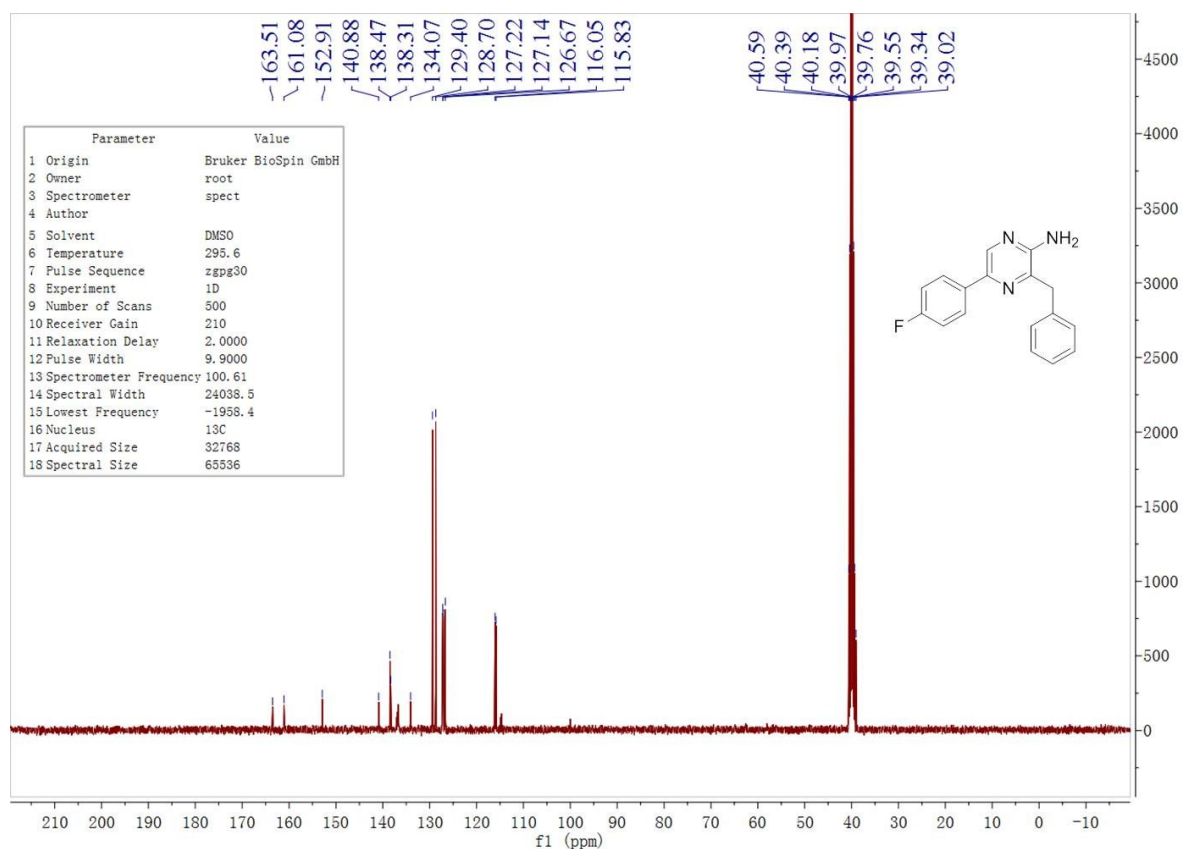
^1H NMR and ^{13}C NMR

1

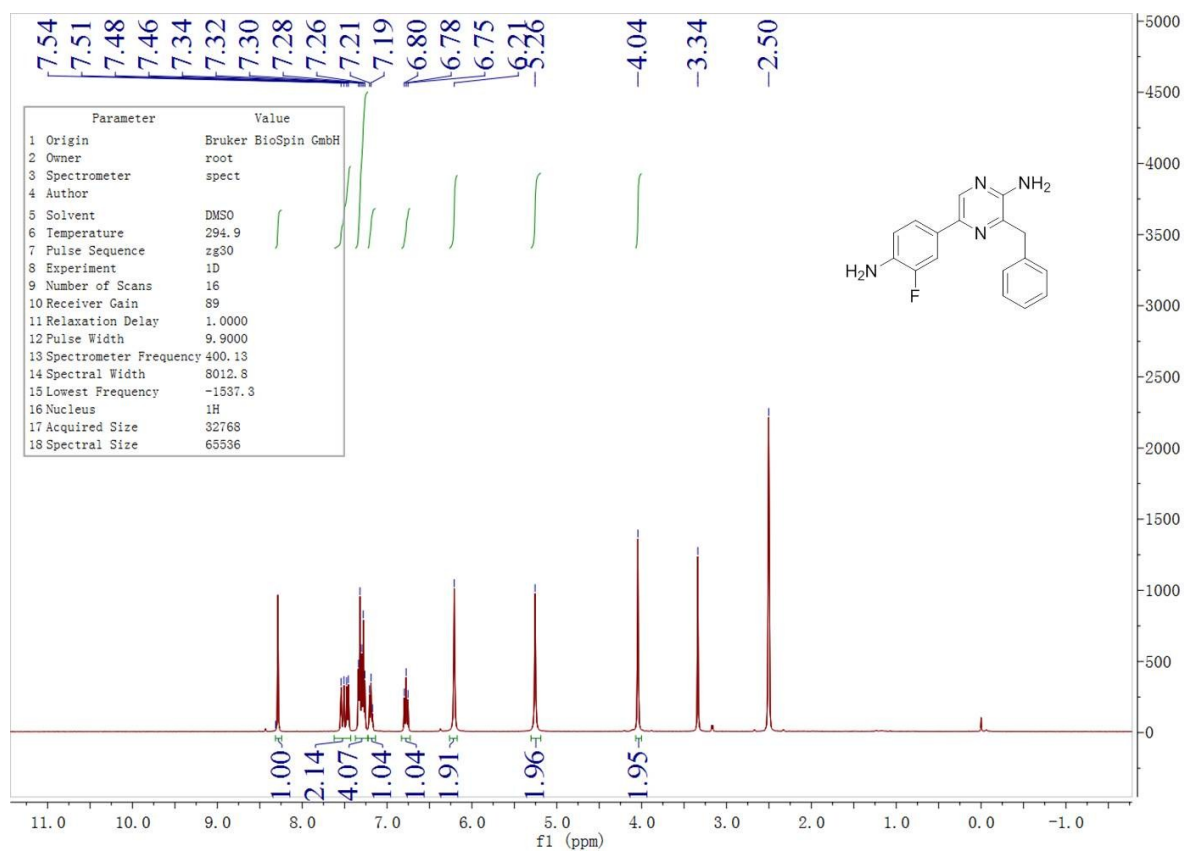


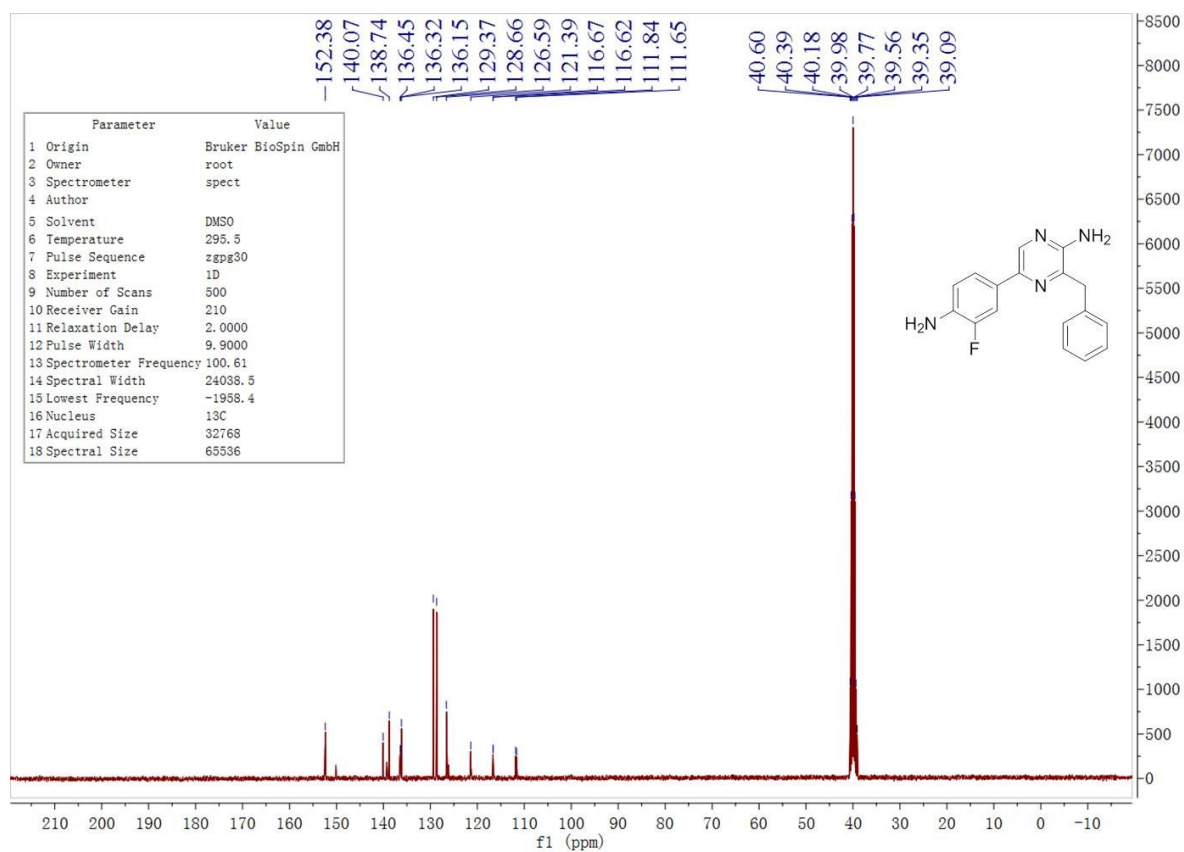
2-1



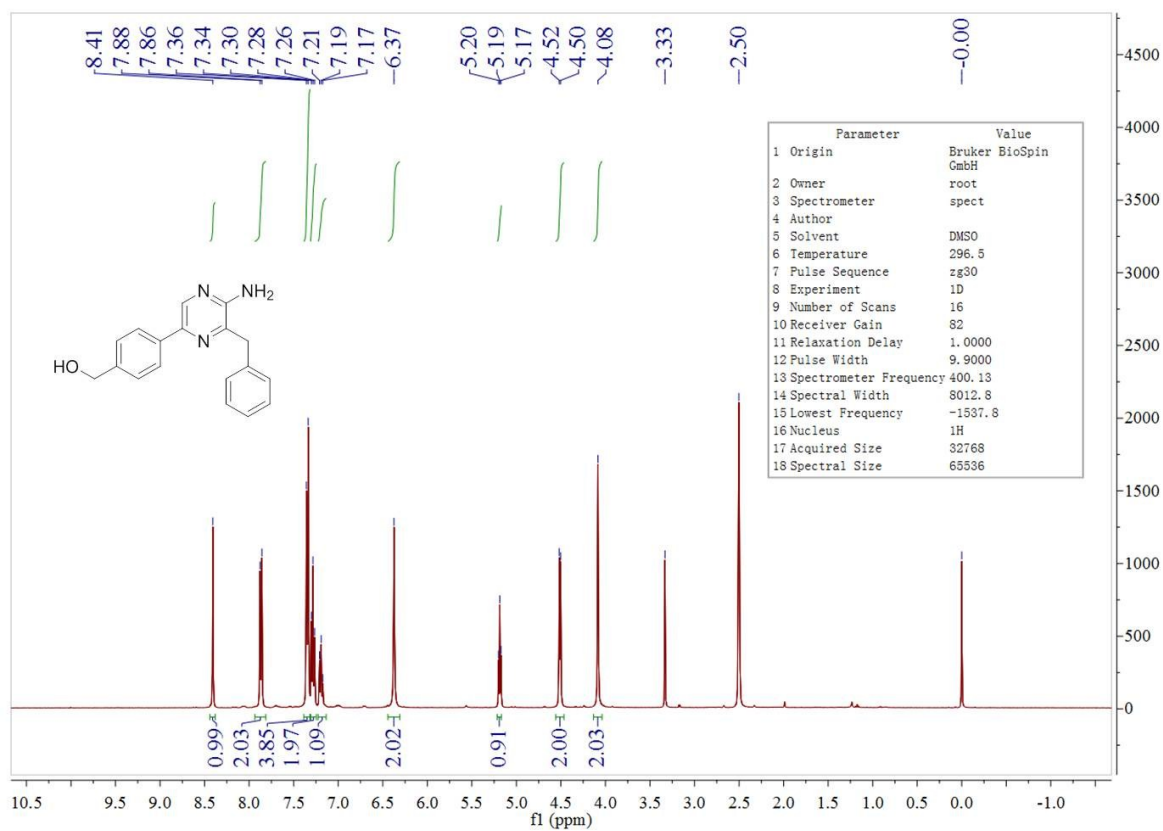


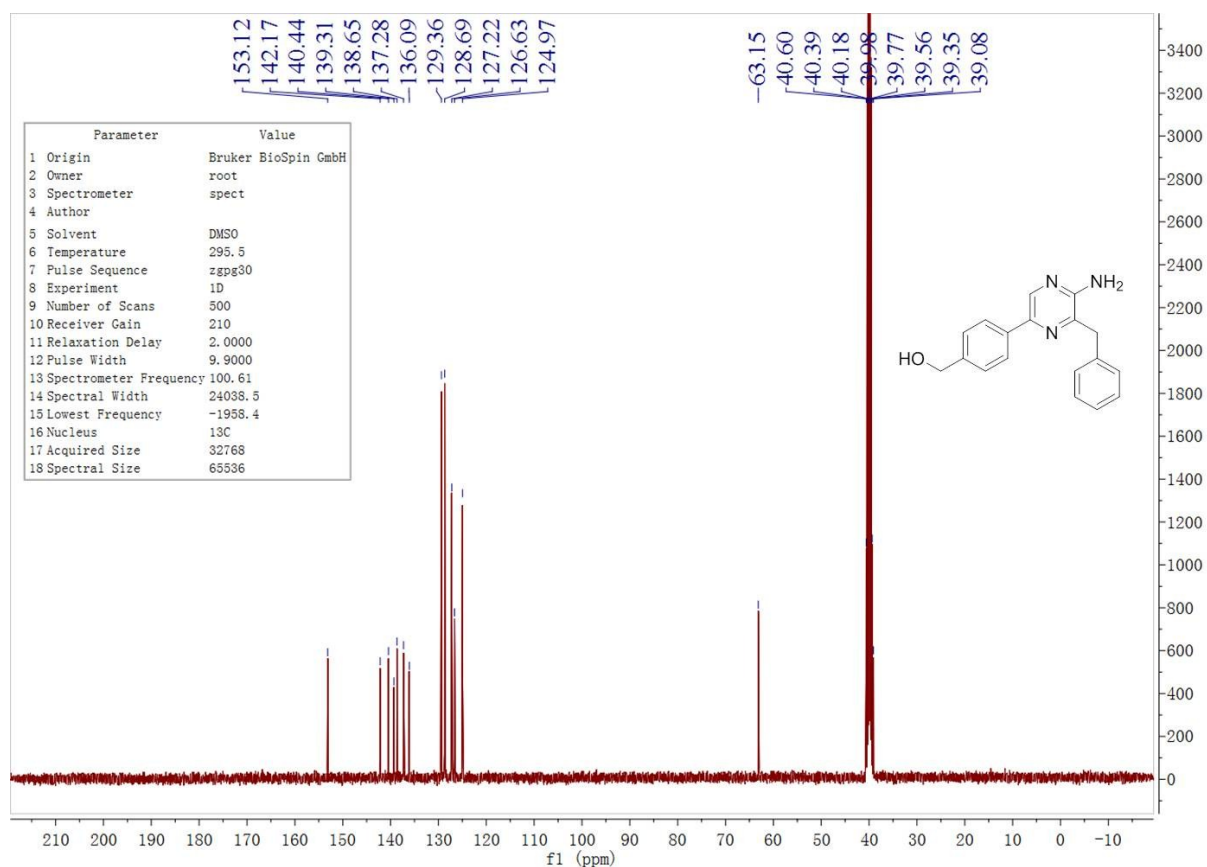
2-2



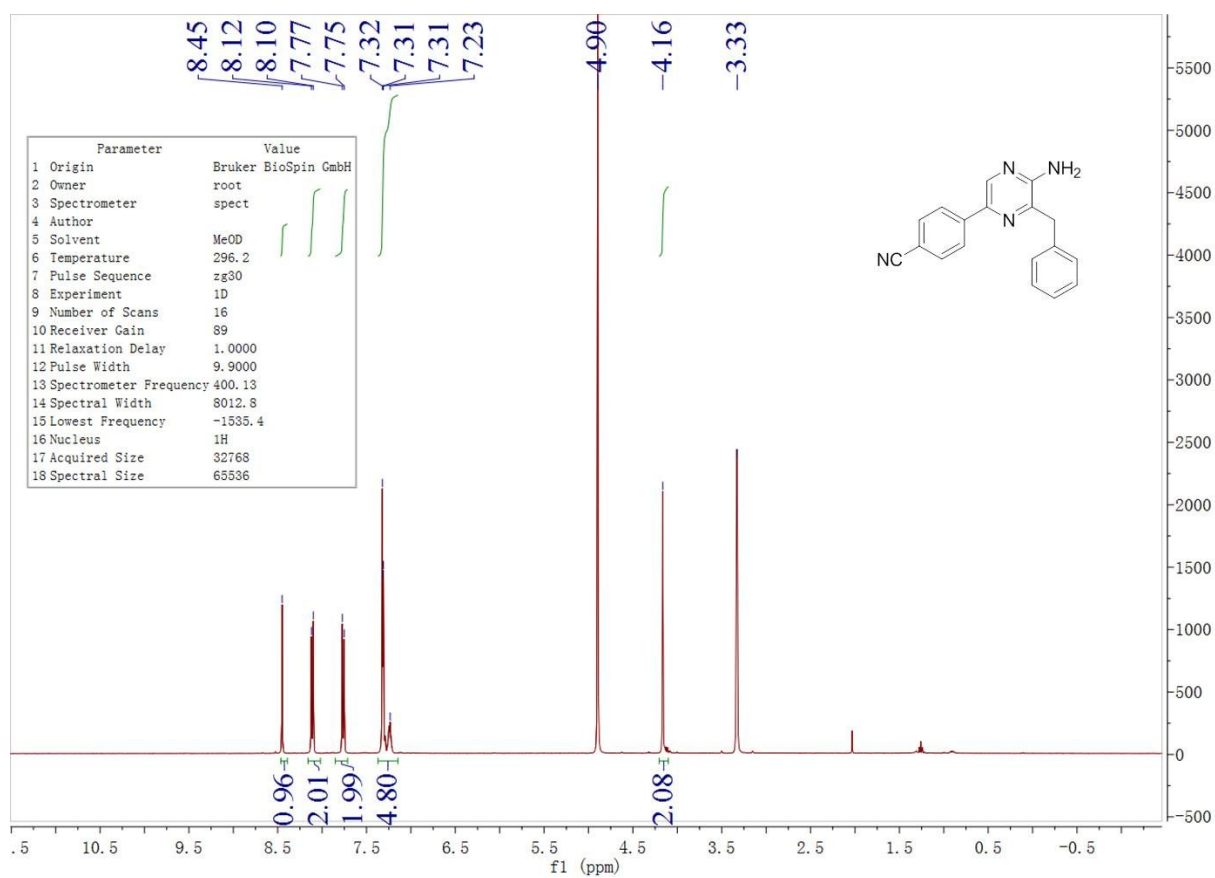


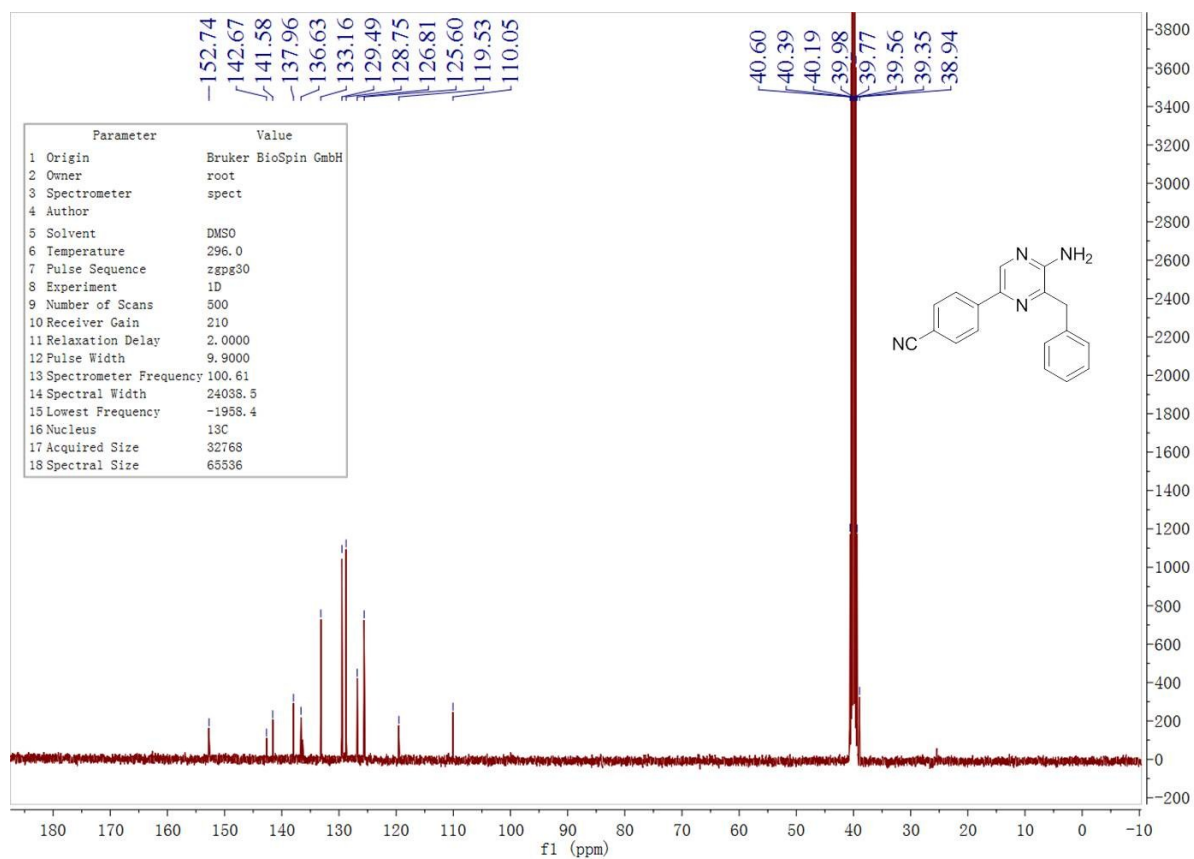
2-3



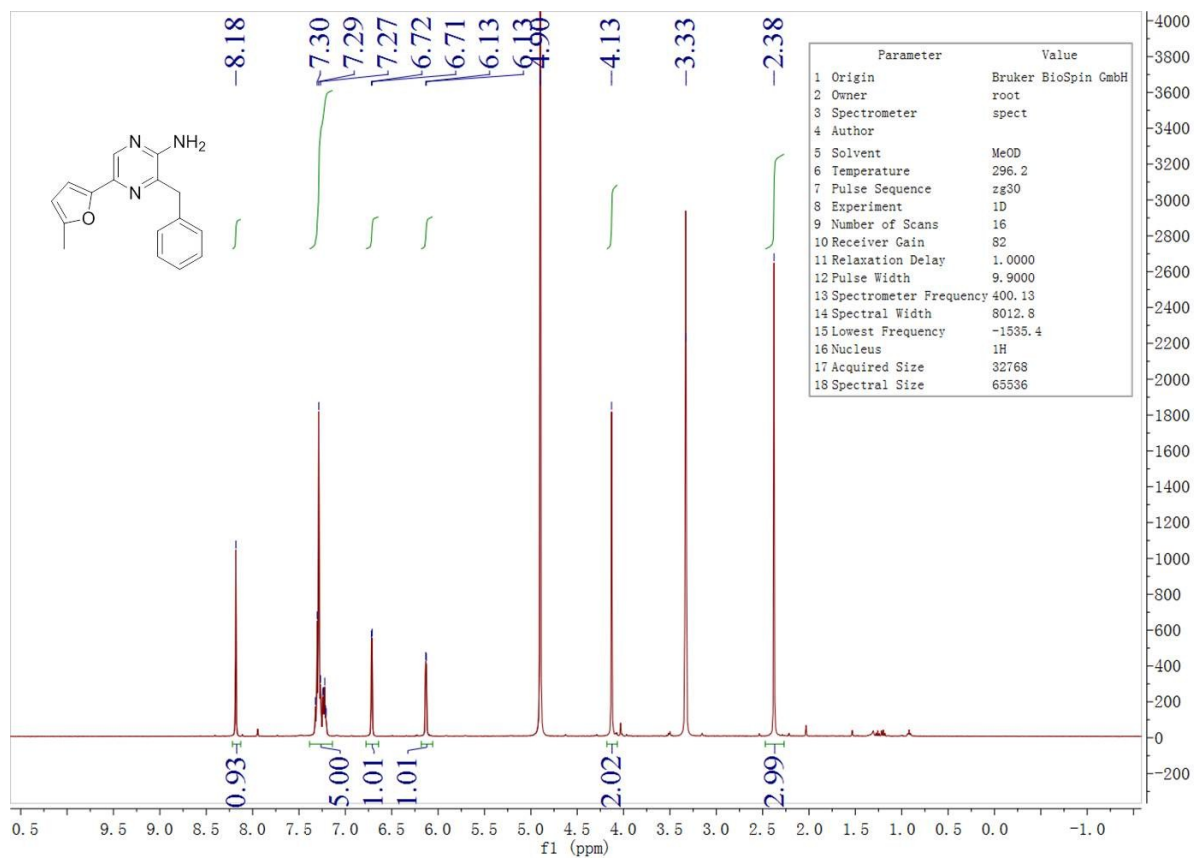


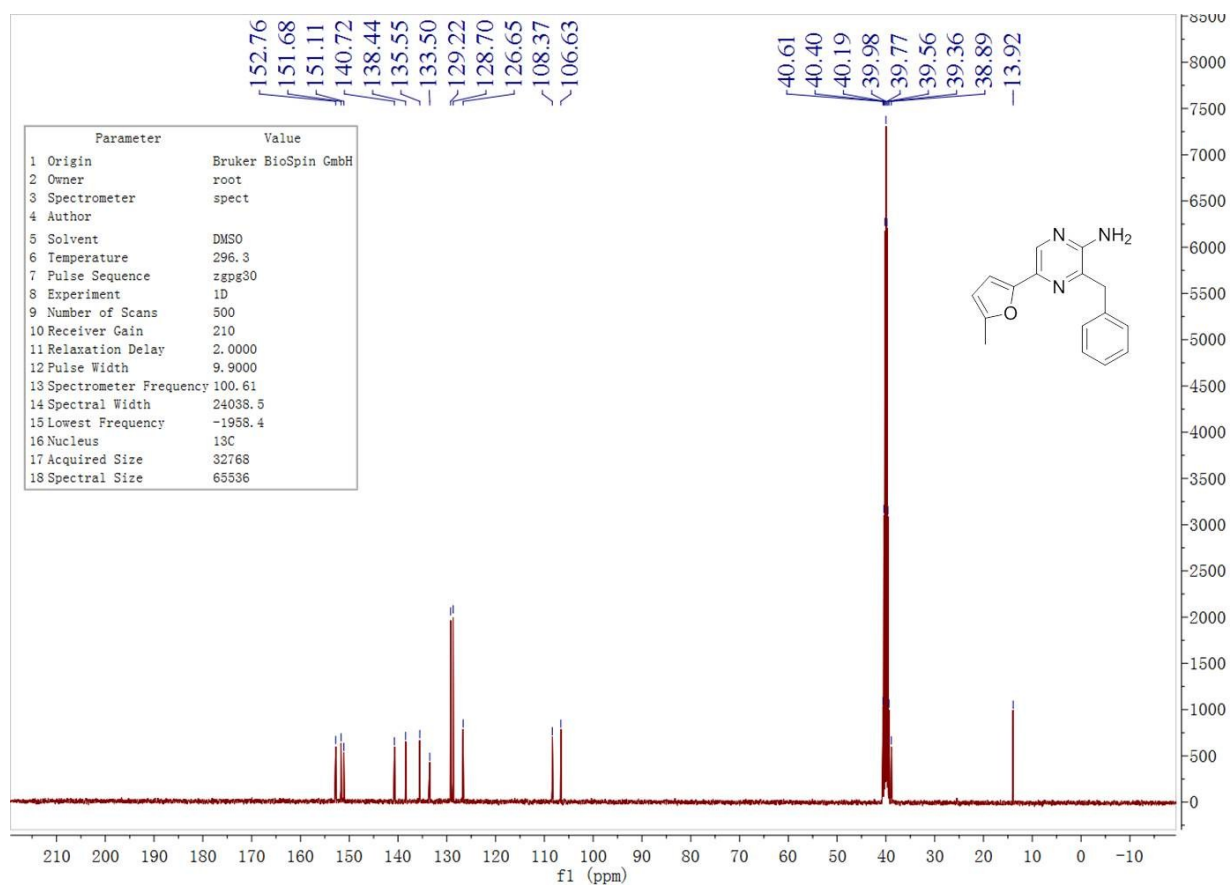
2-4



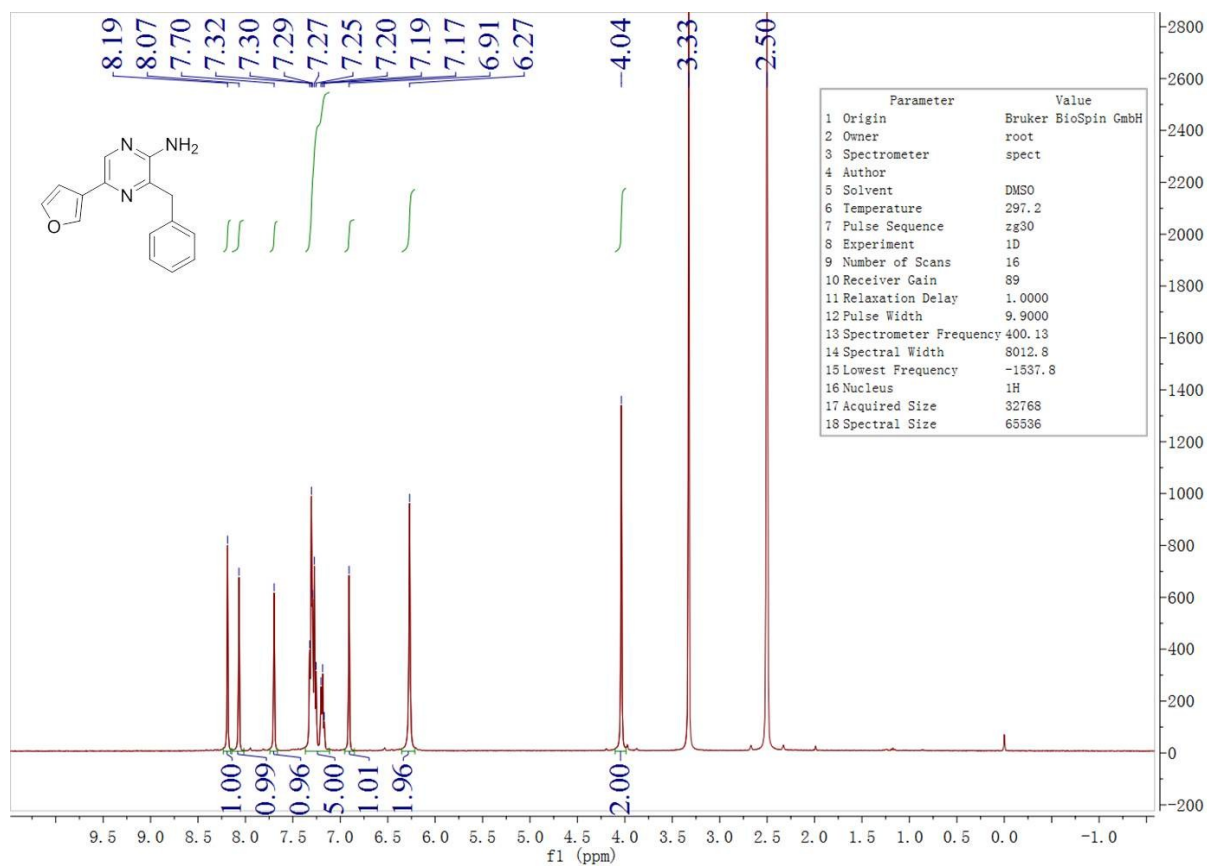


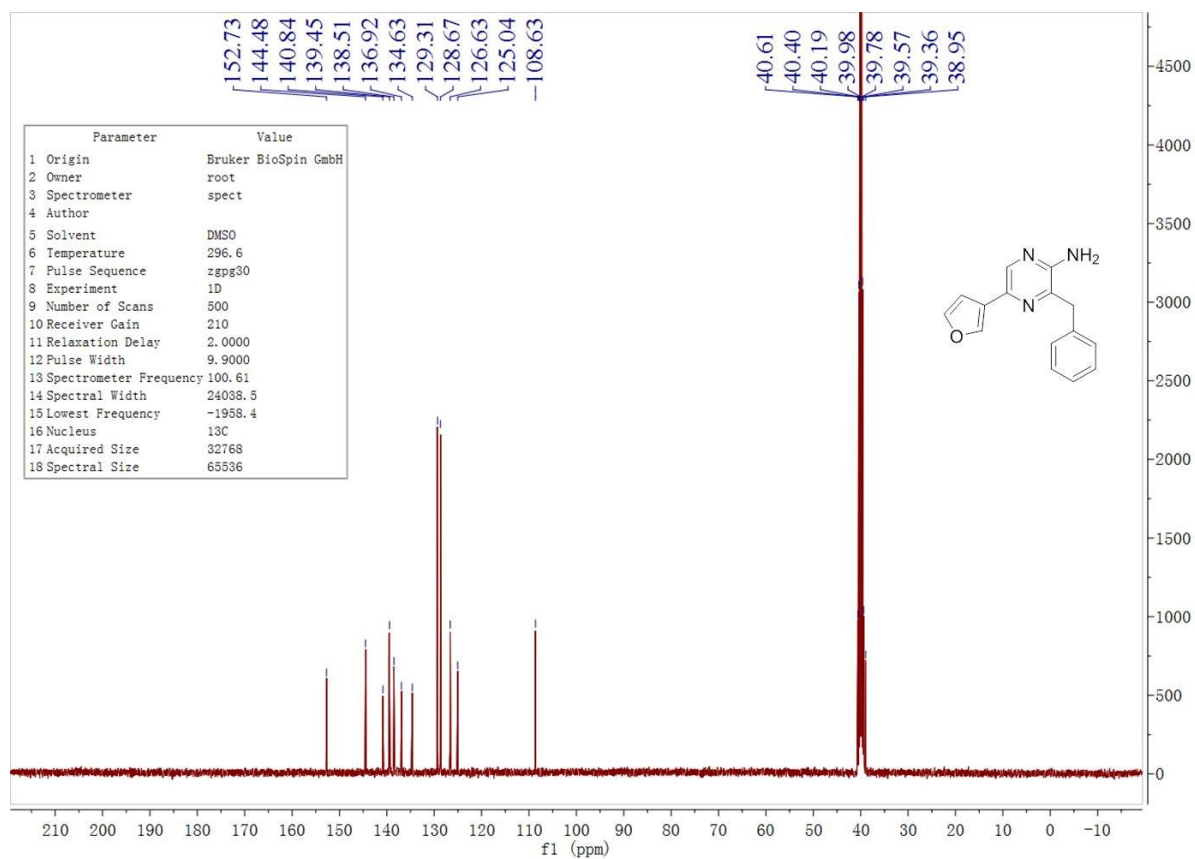
2-5



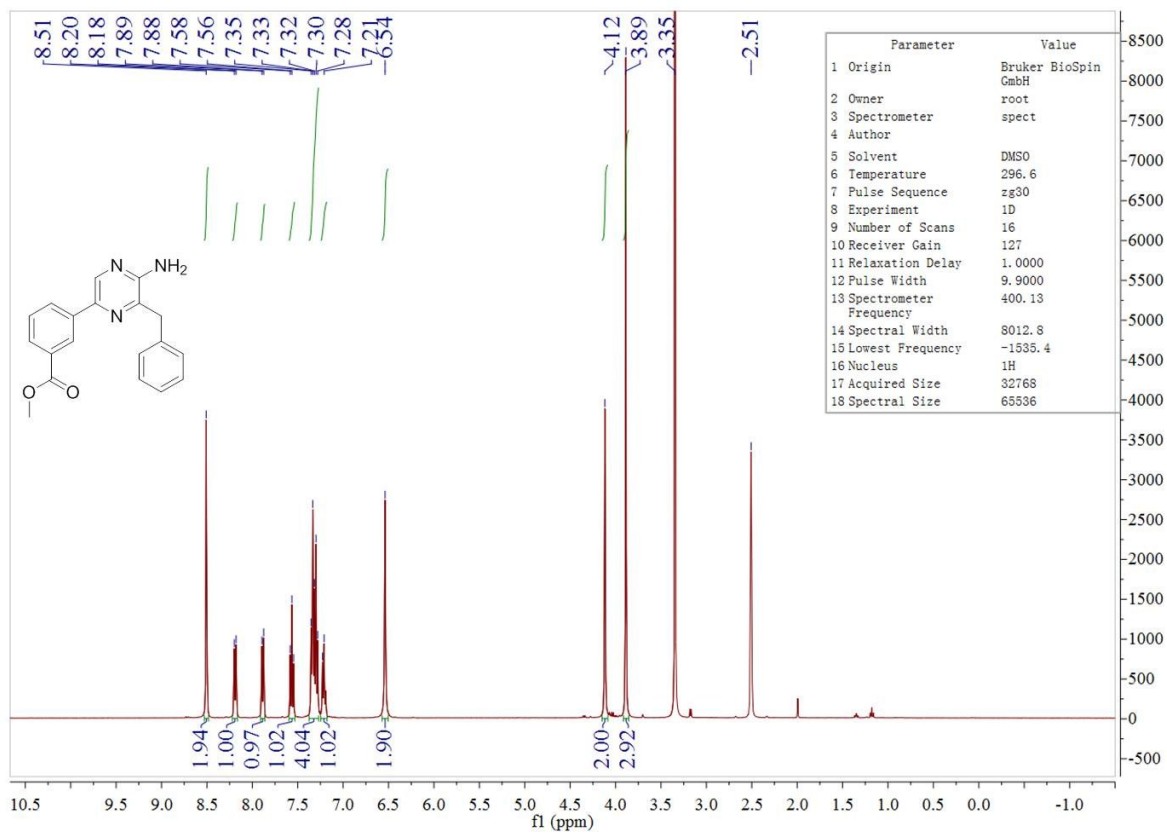


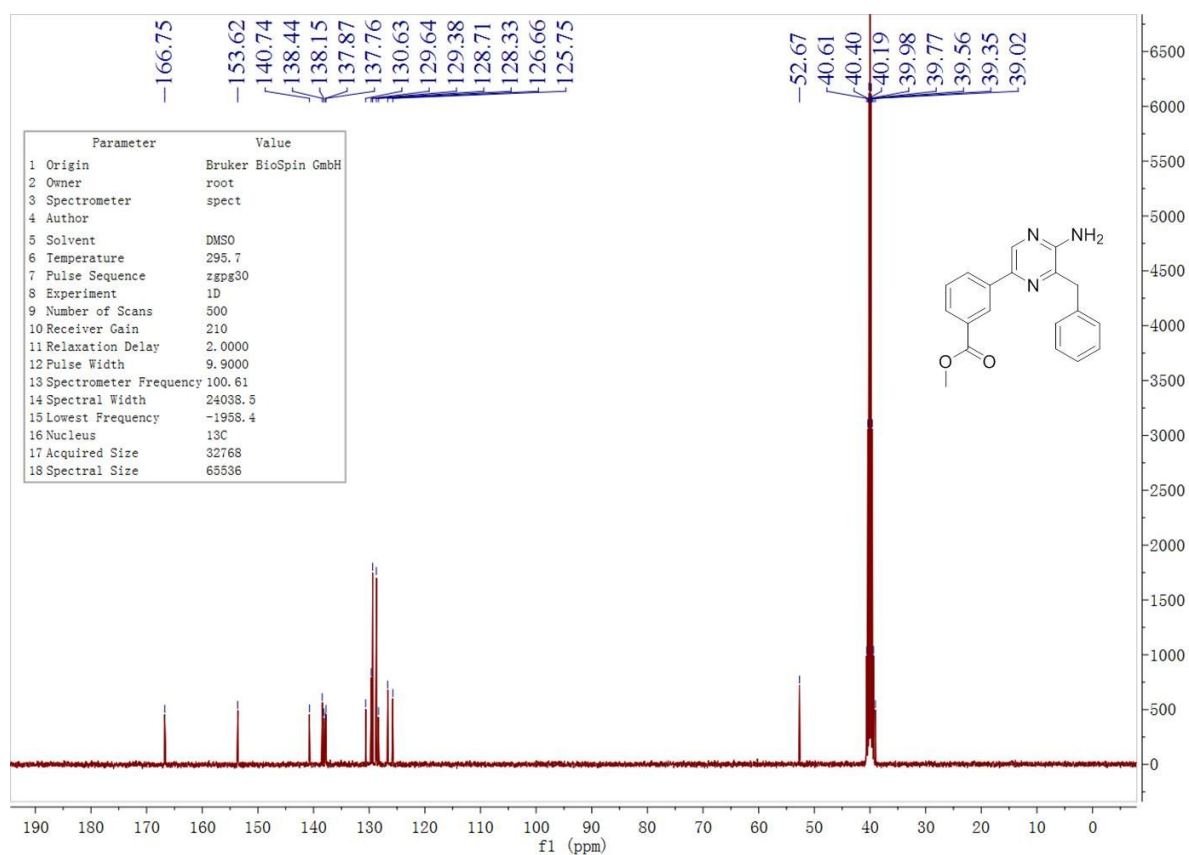
2-6



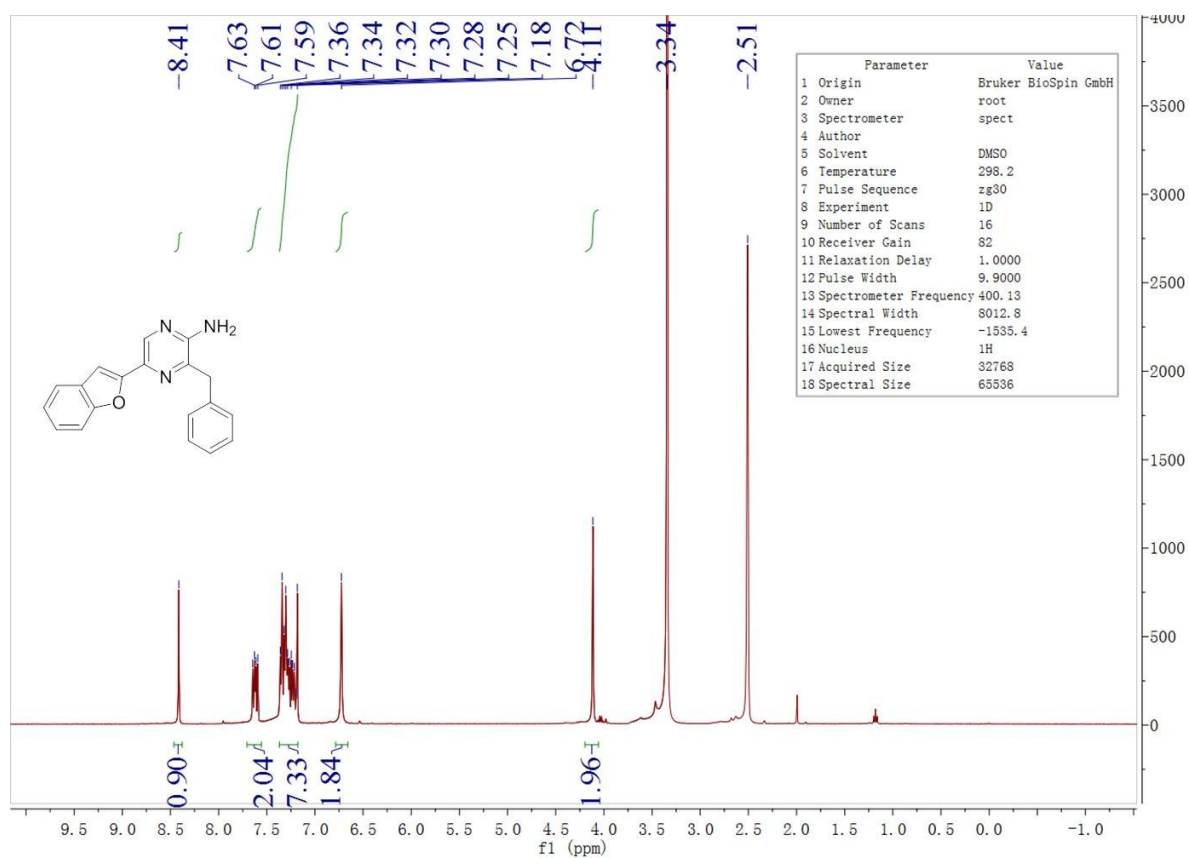


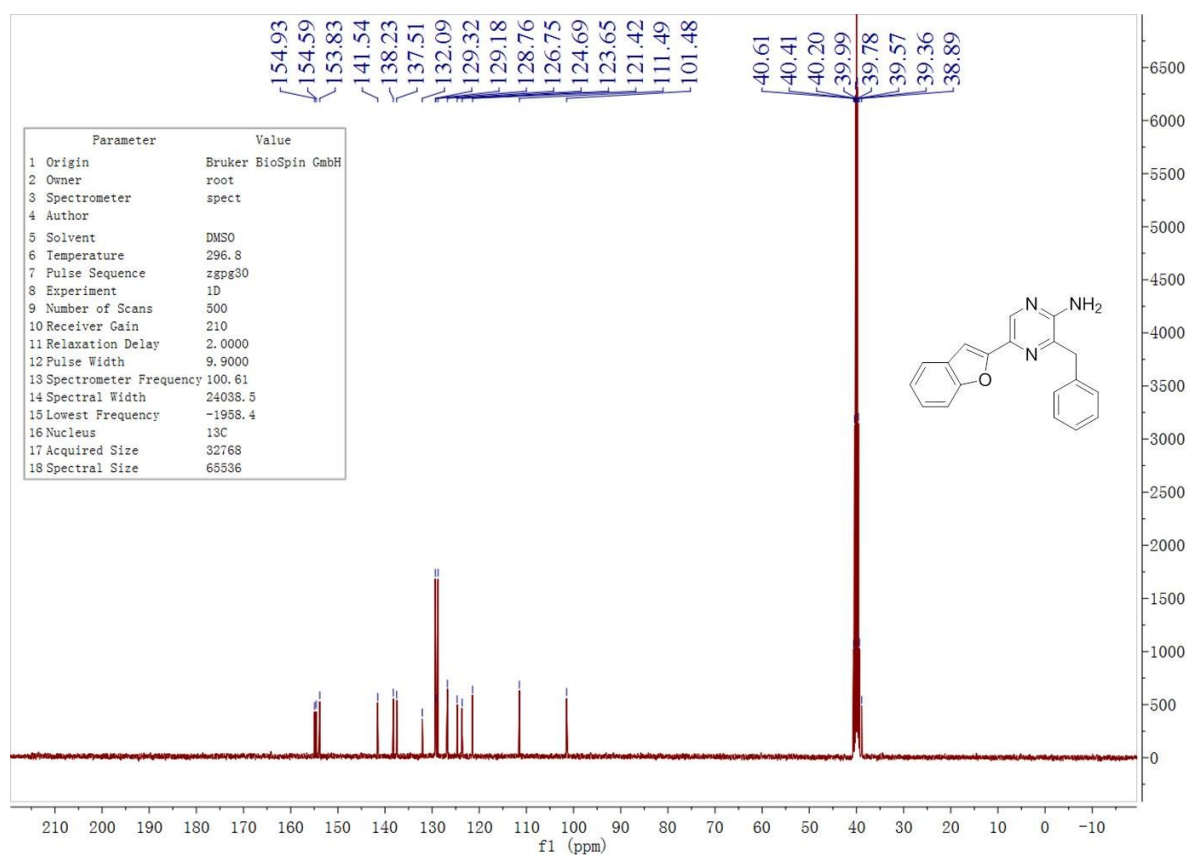
2-7



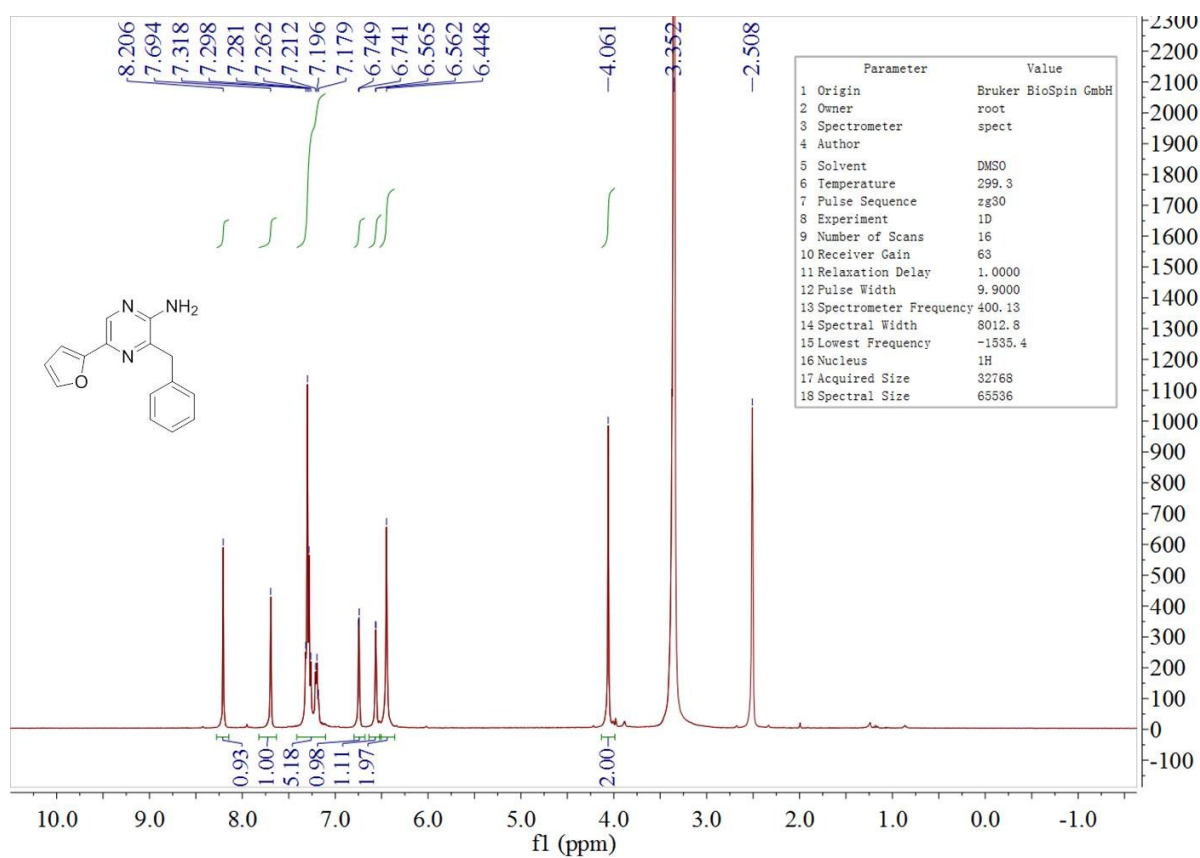


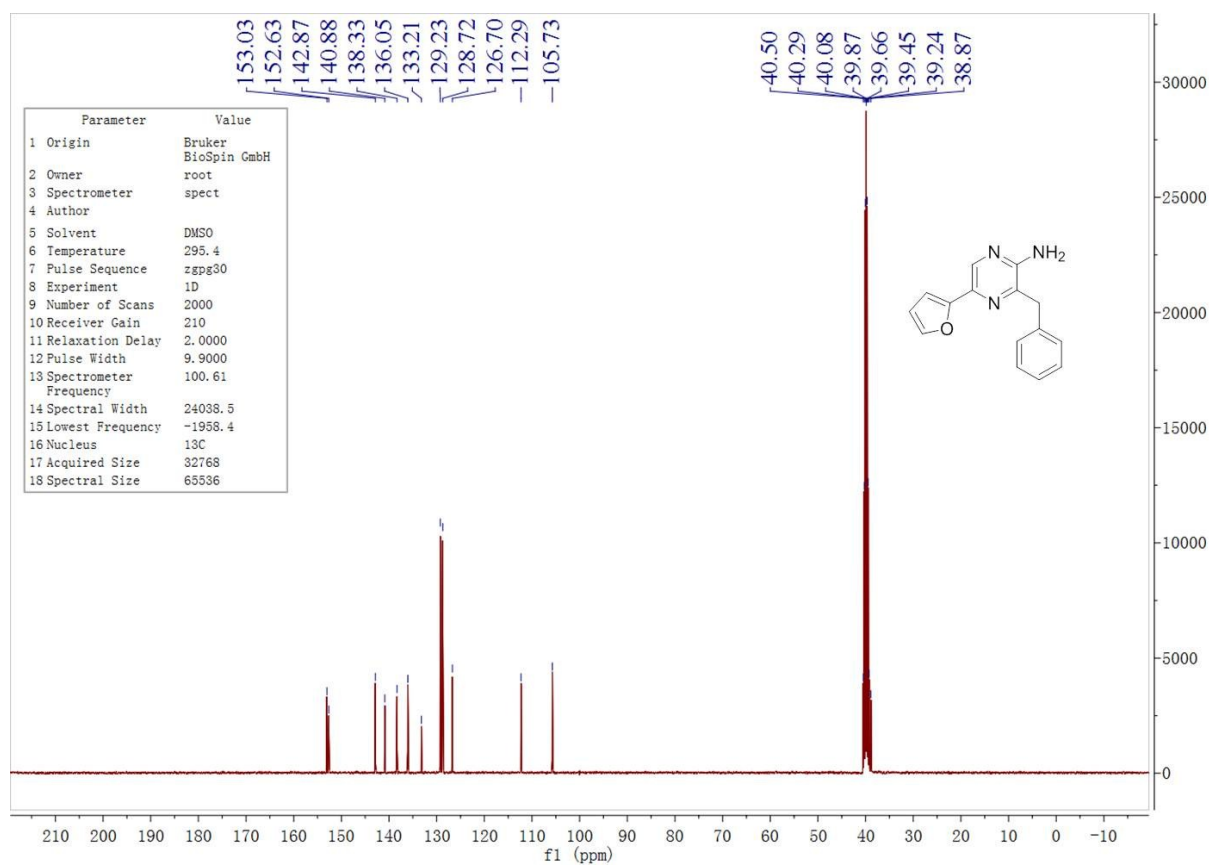
2-8



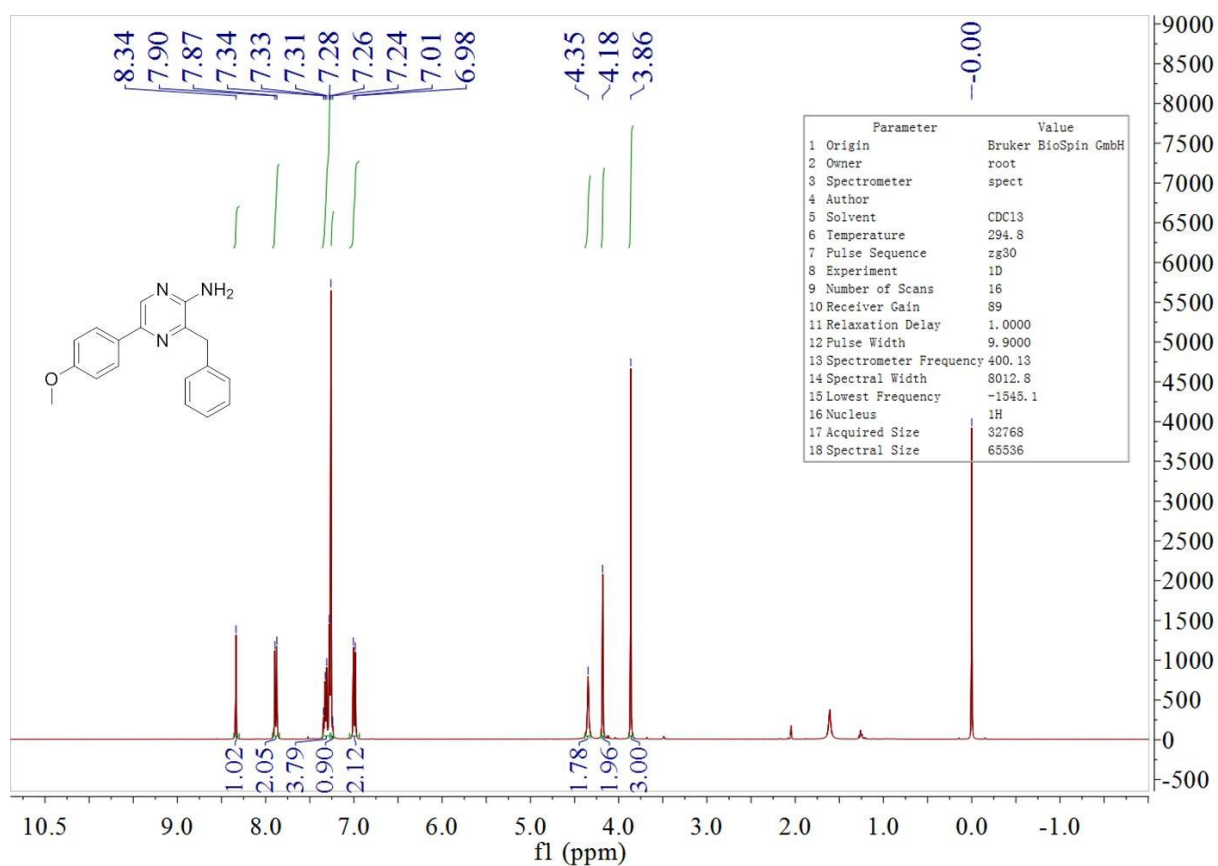


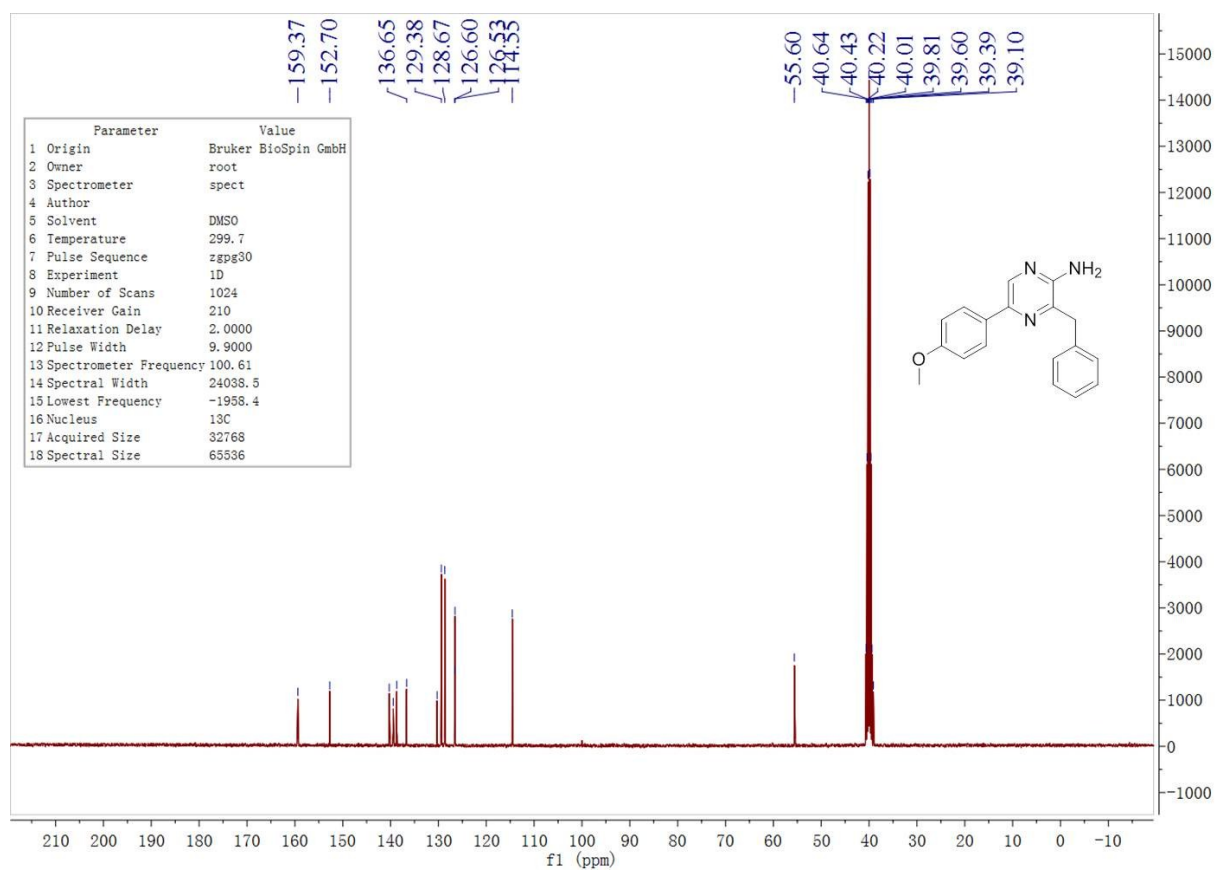
2-9



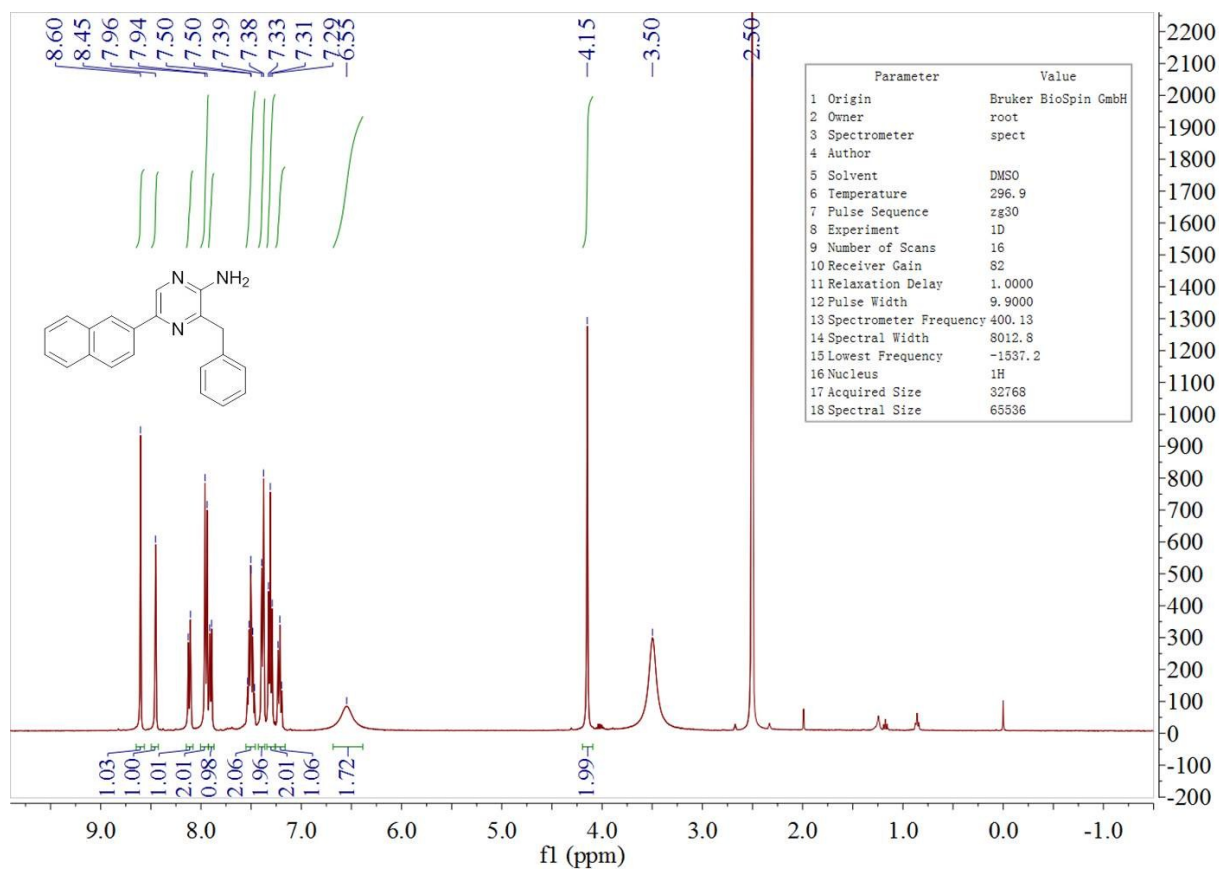


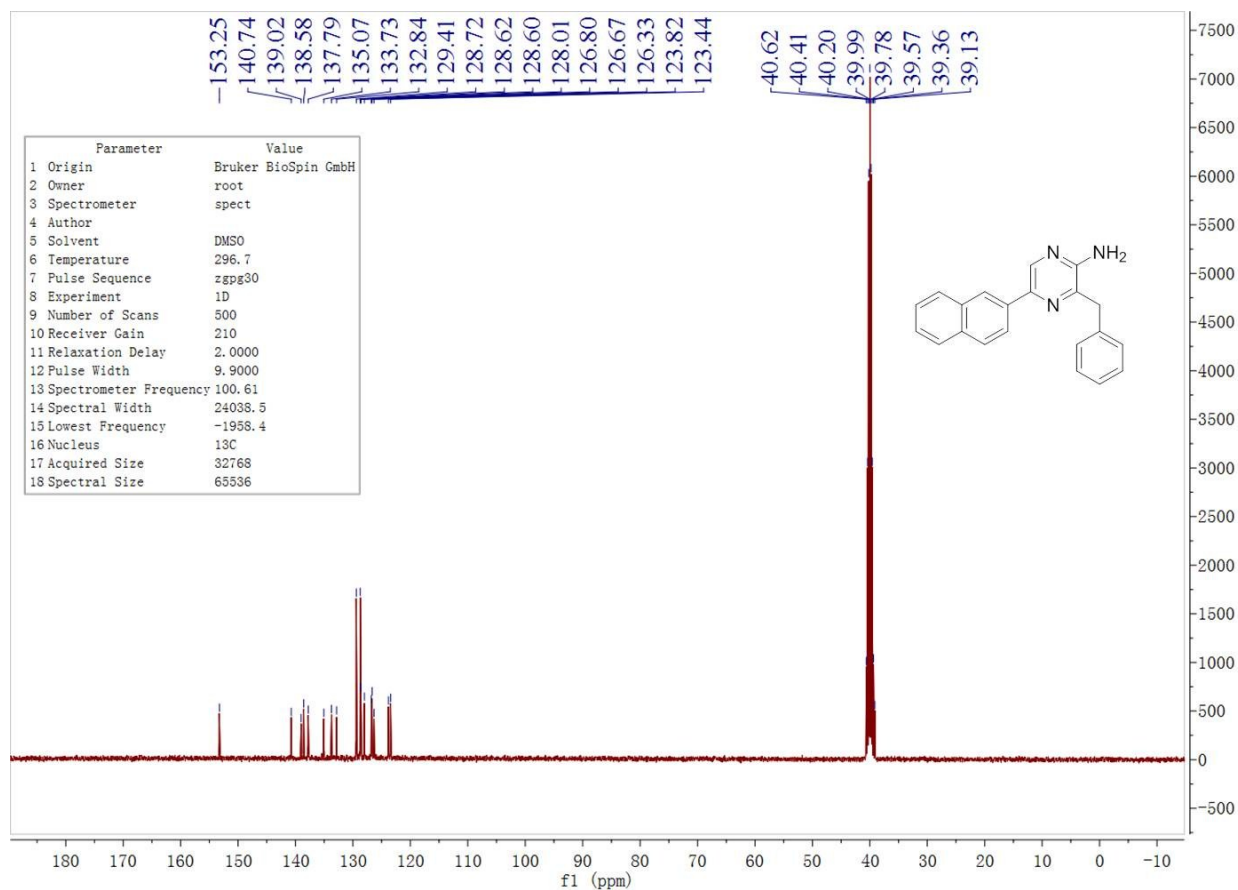
2-10



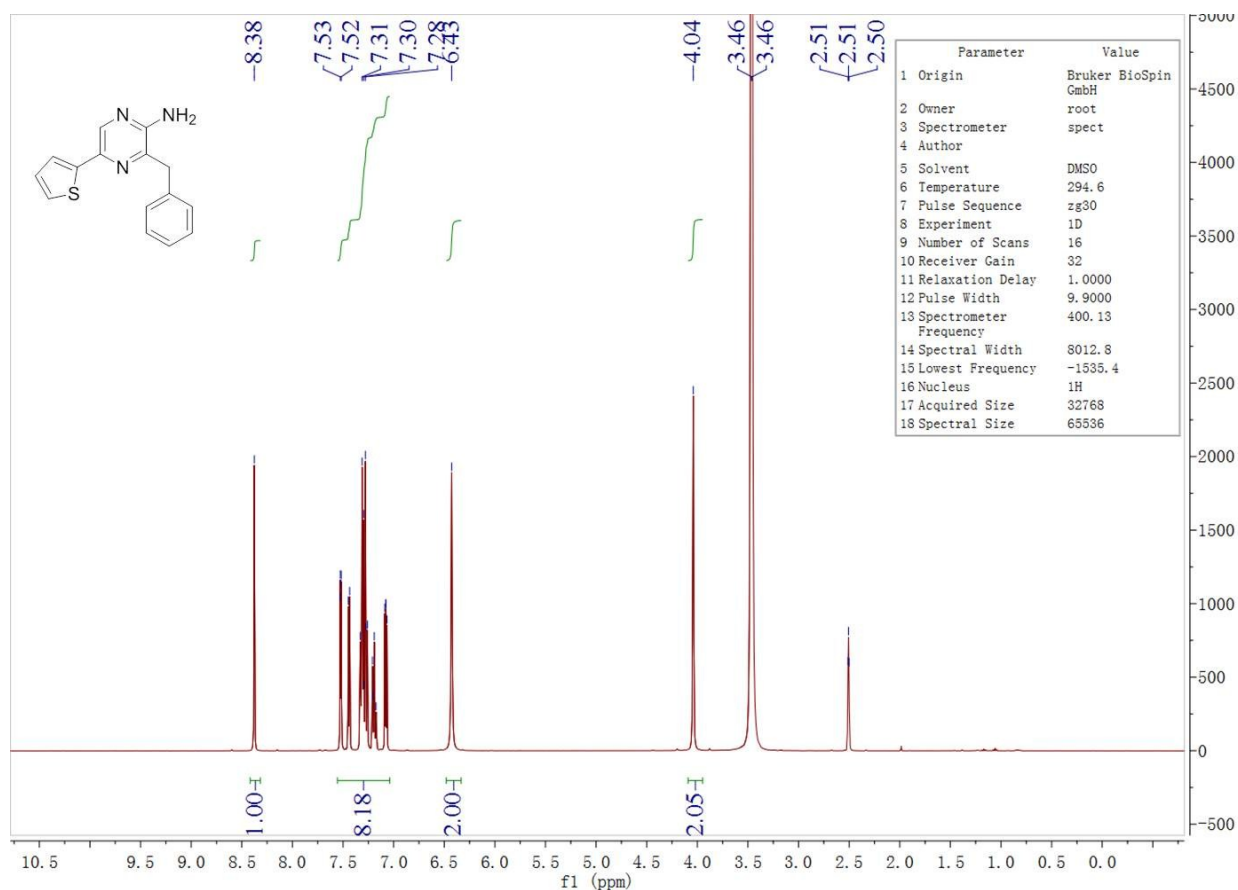


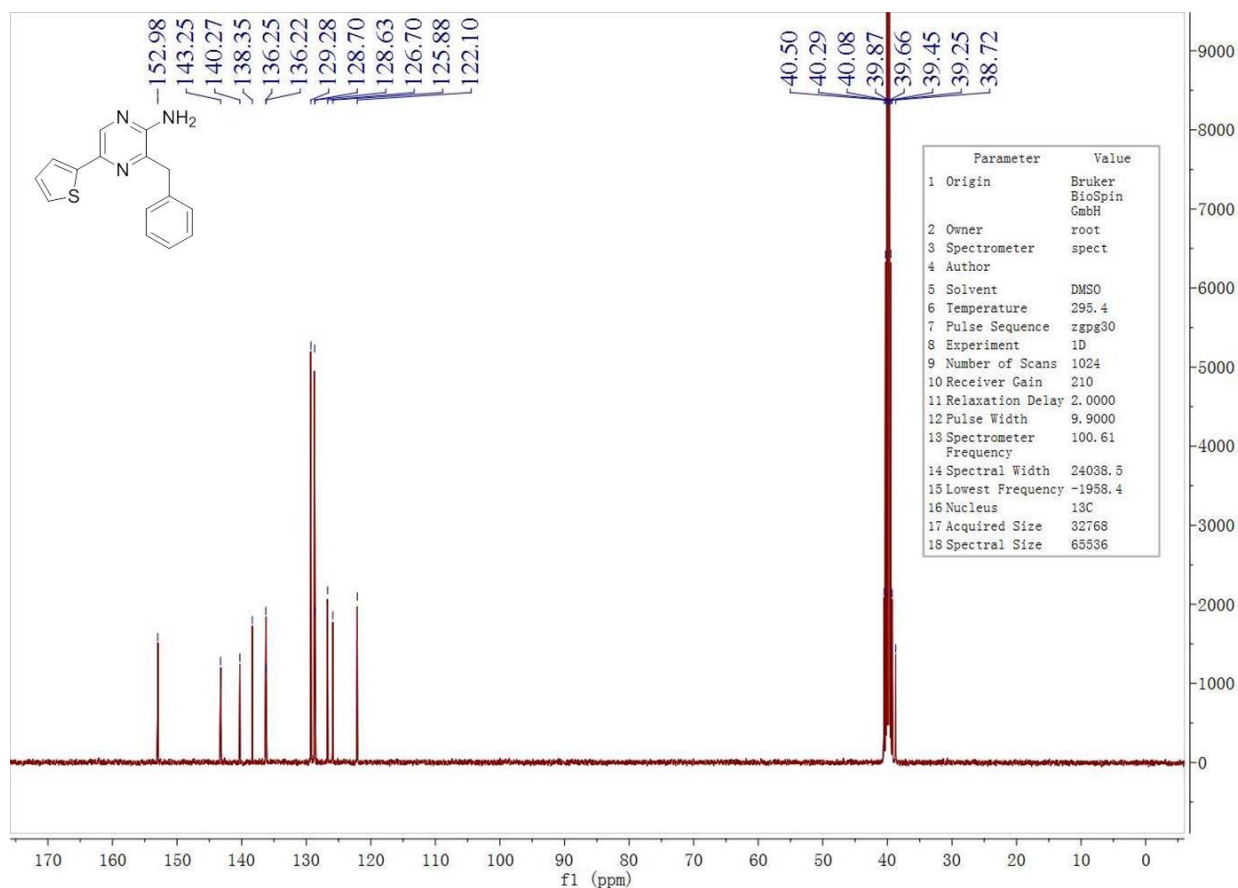
2-11



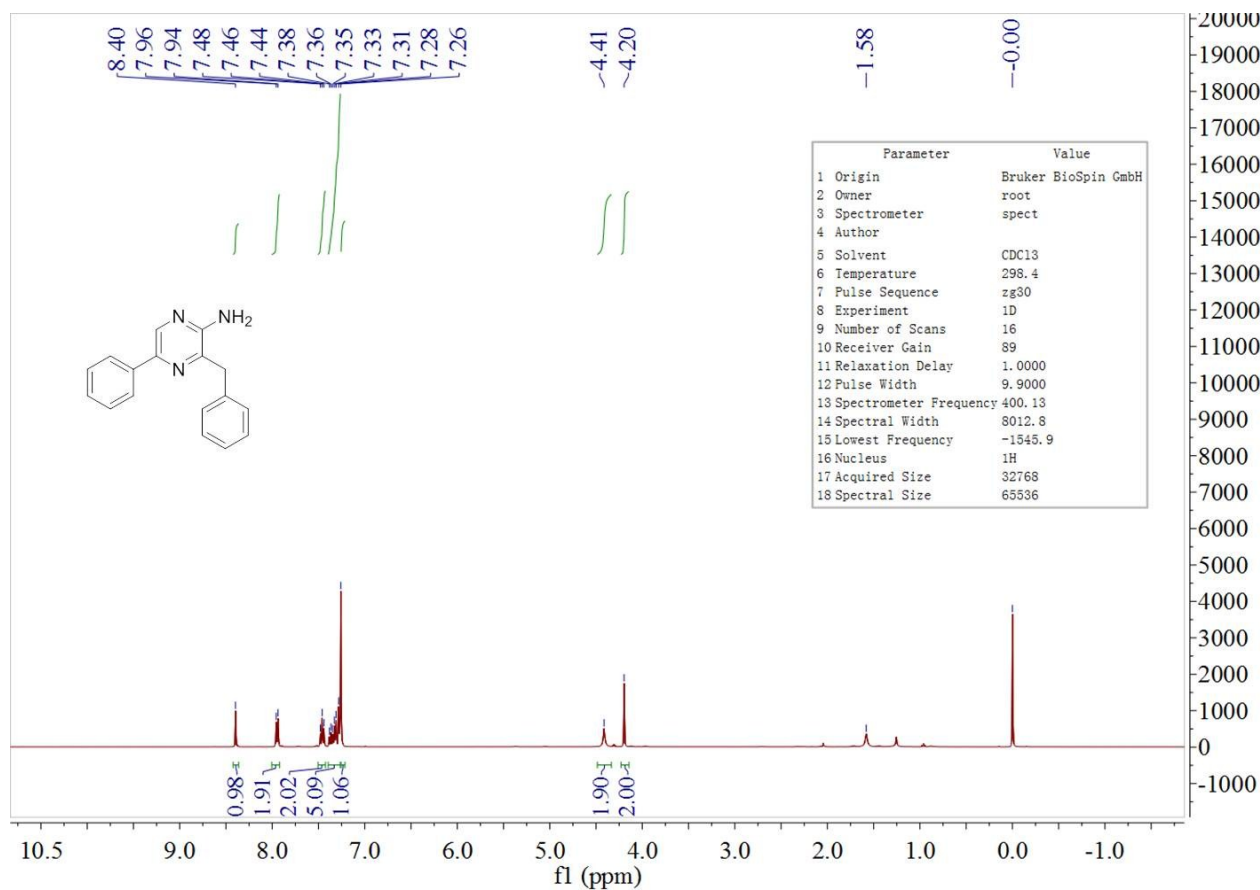


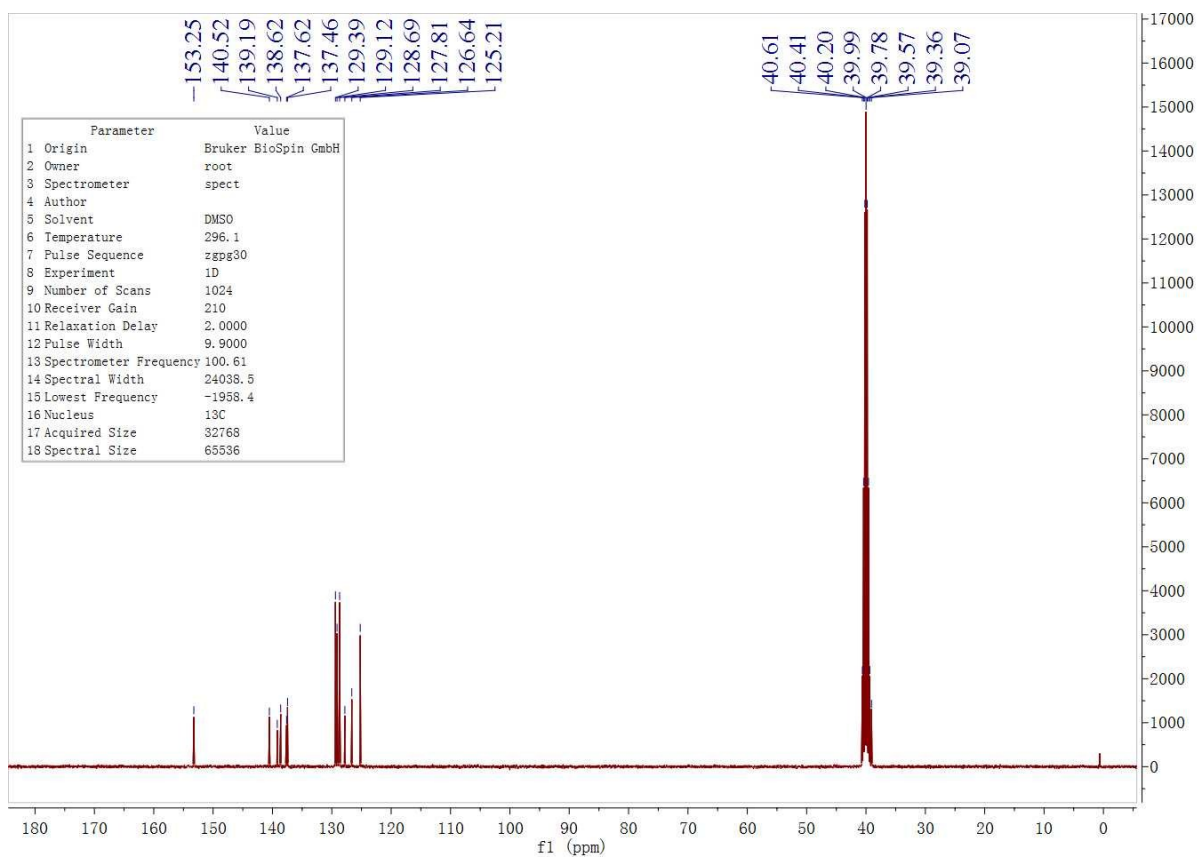
2-12



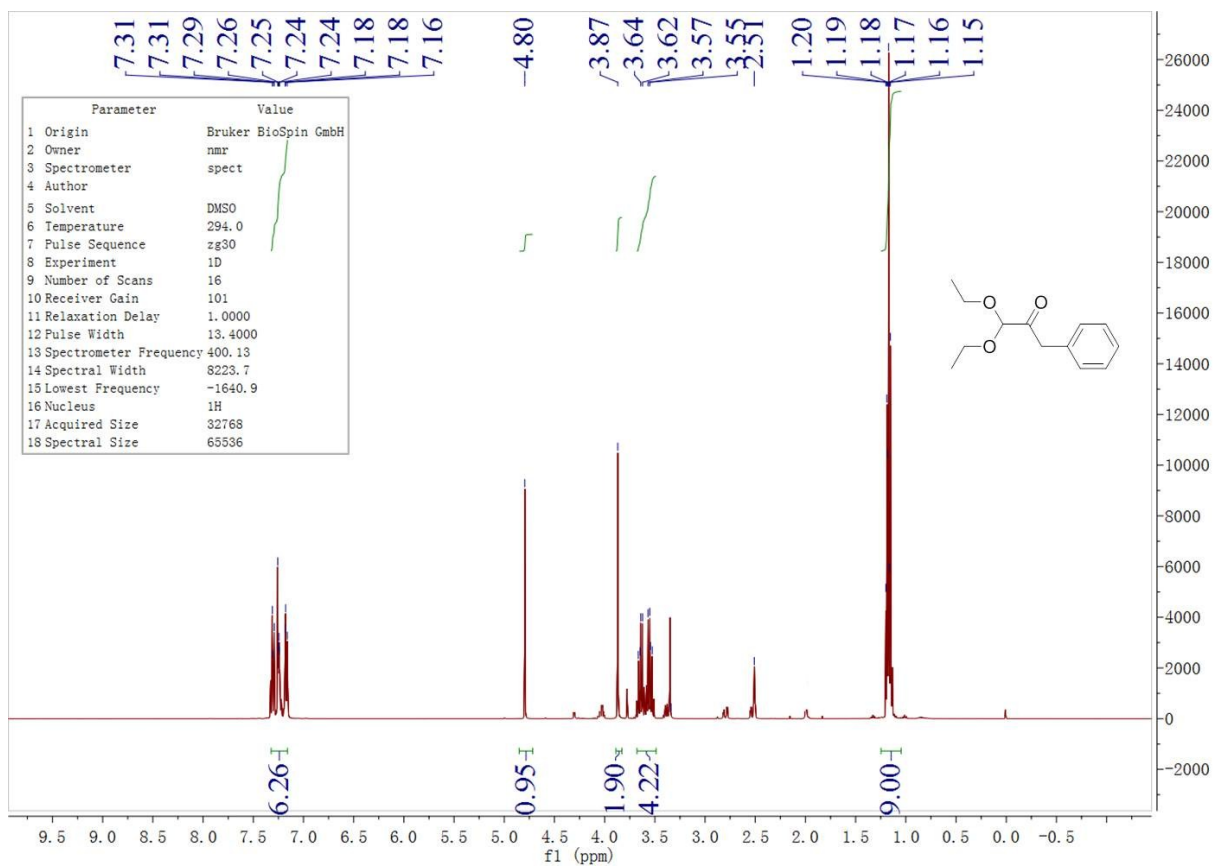


2-13

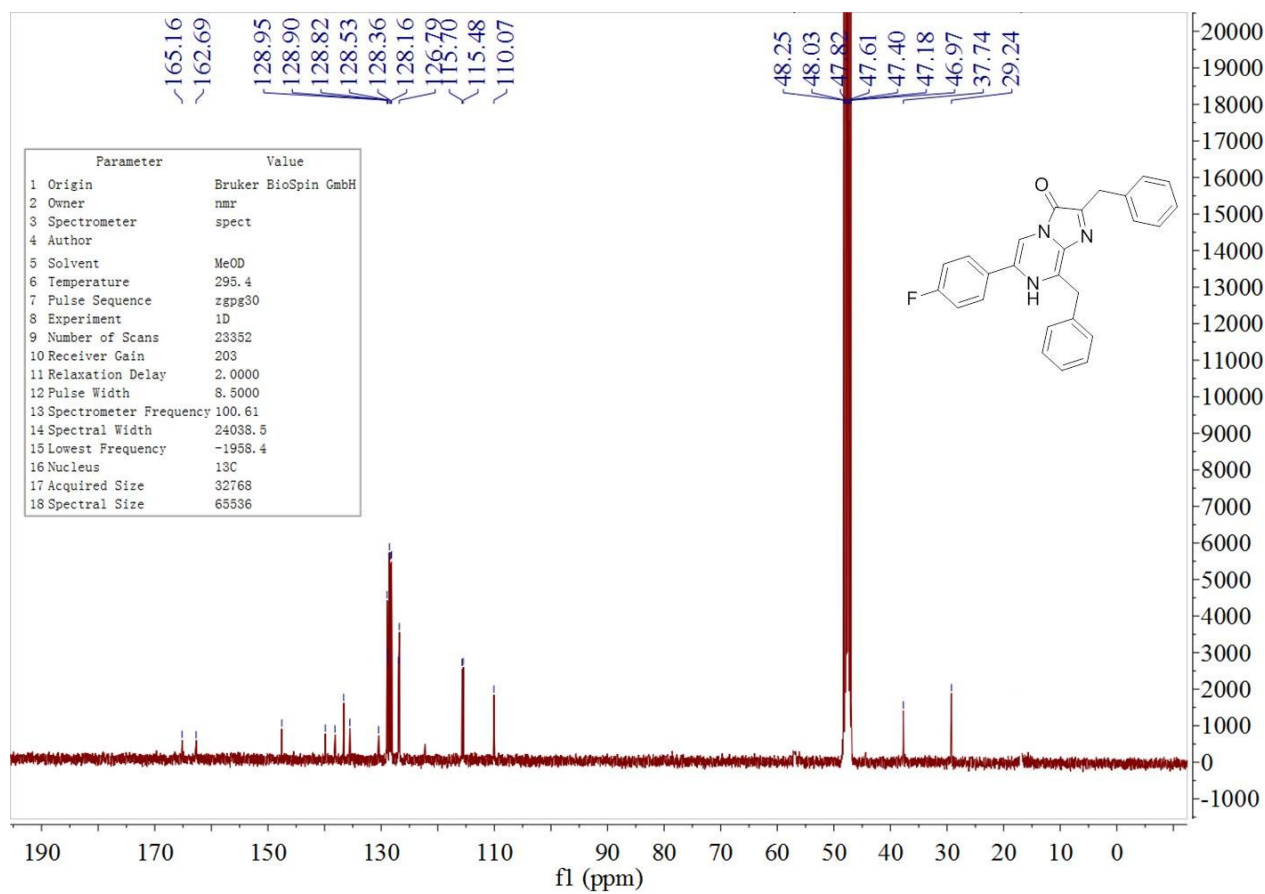
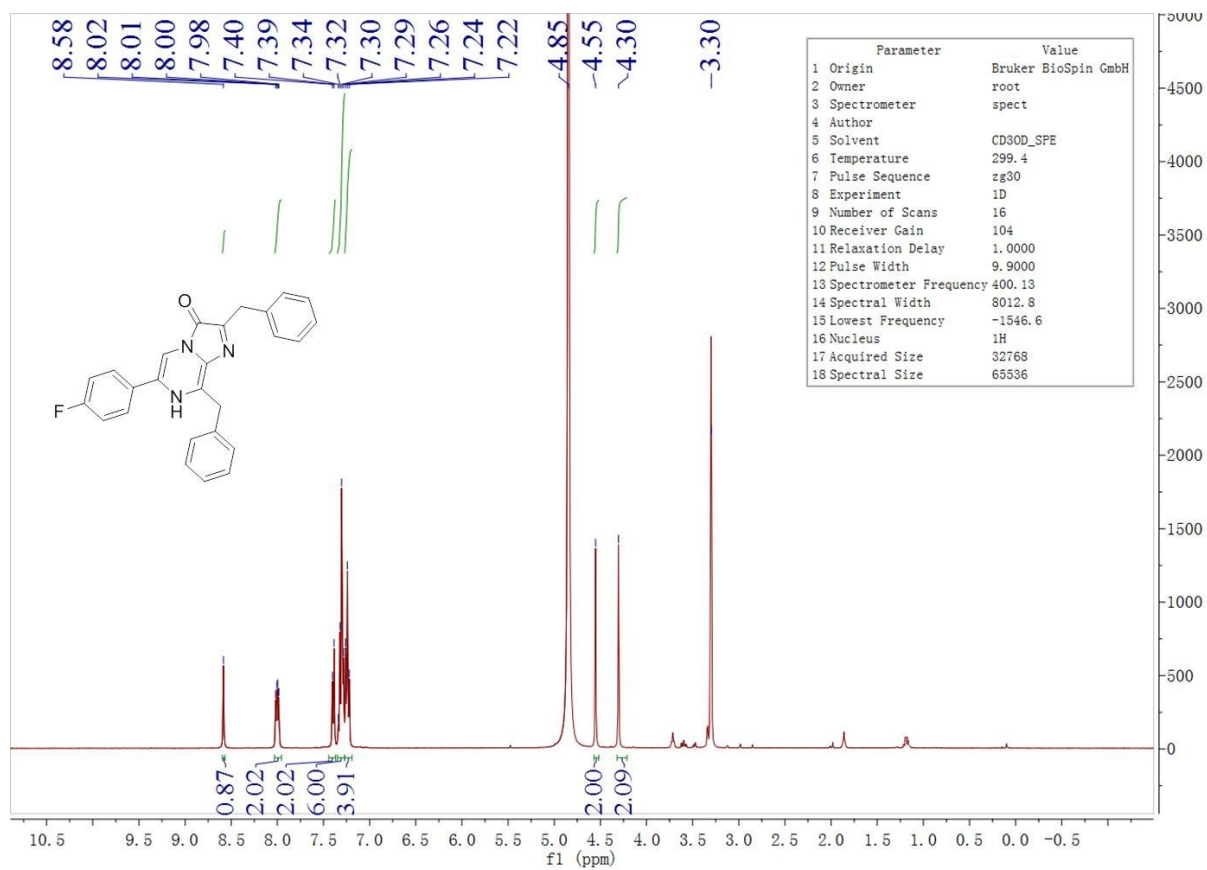




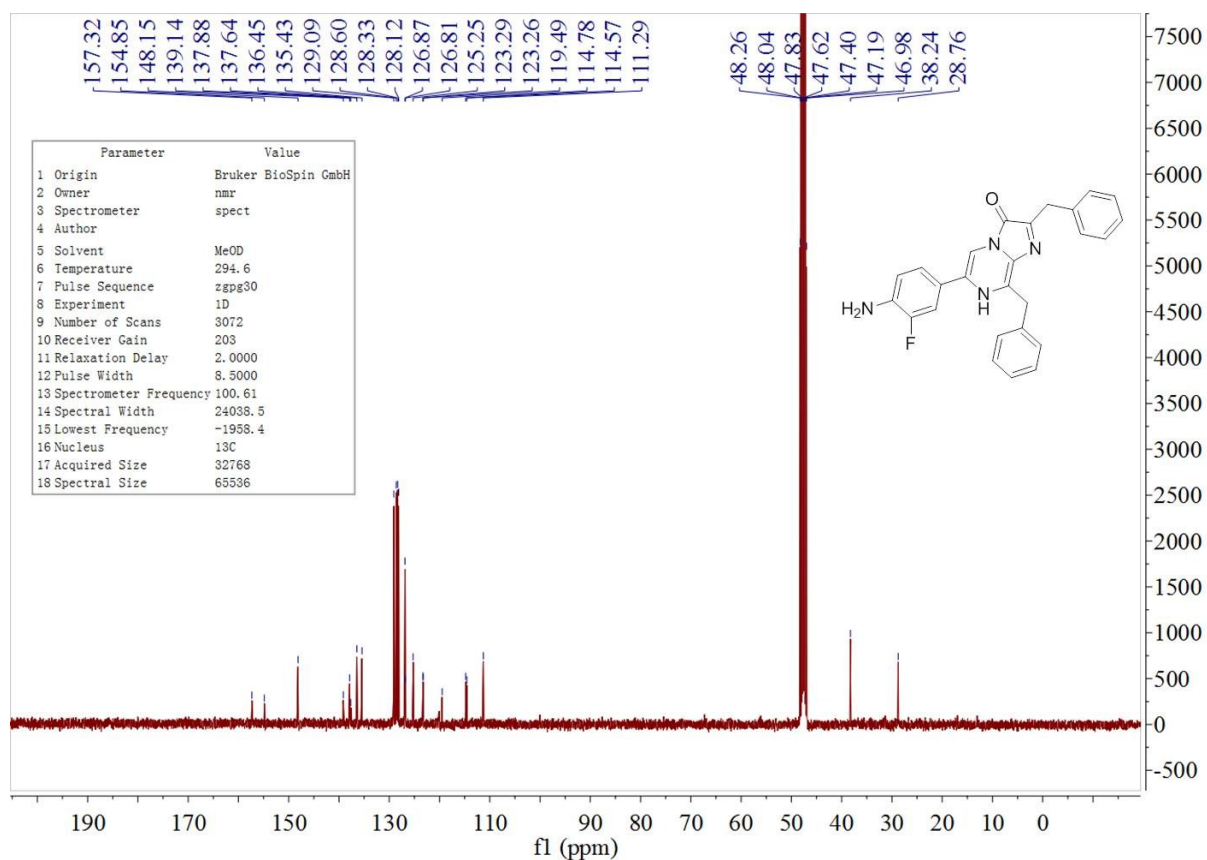
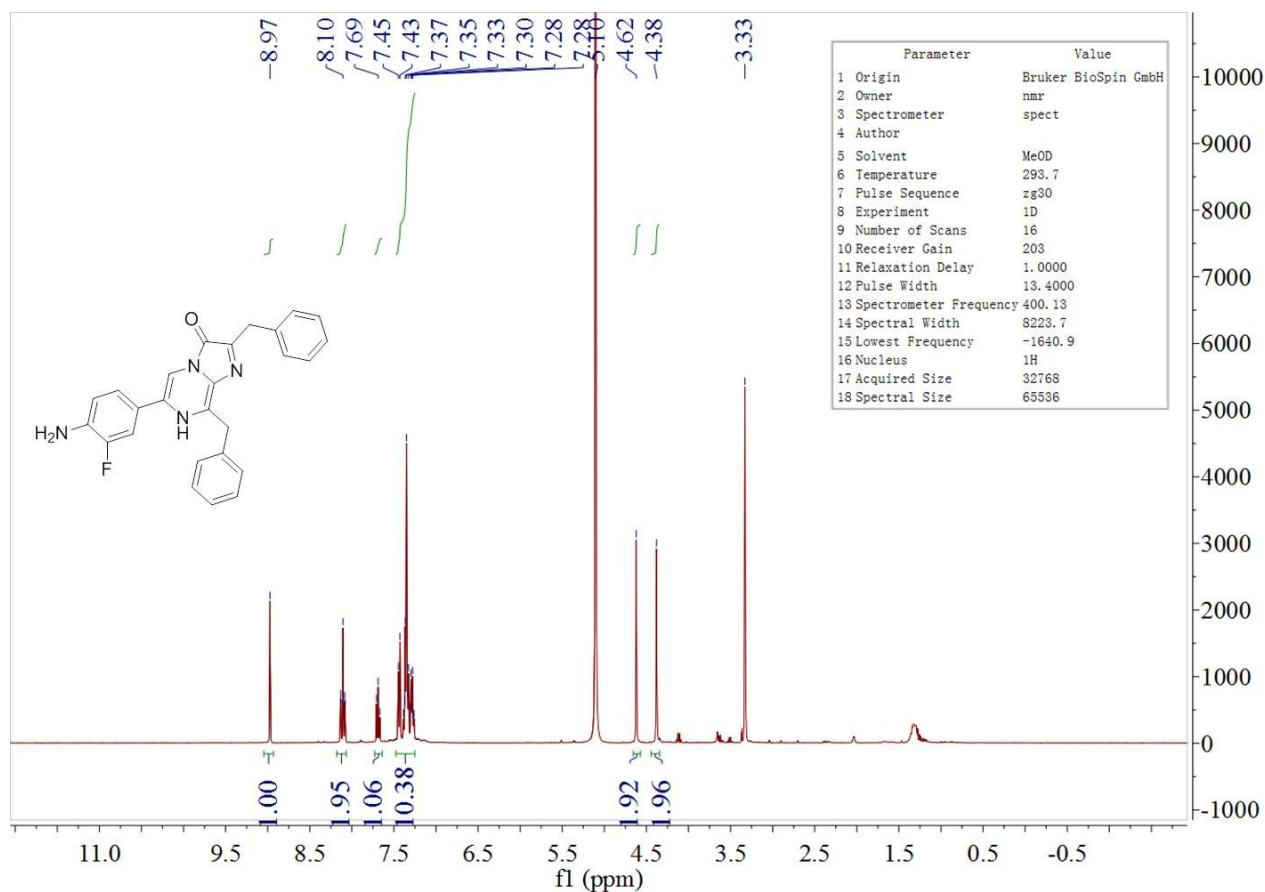
3



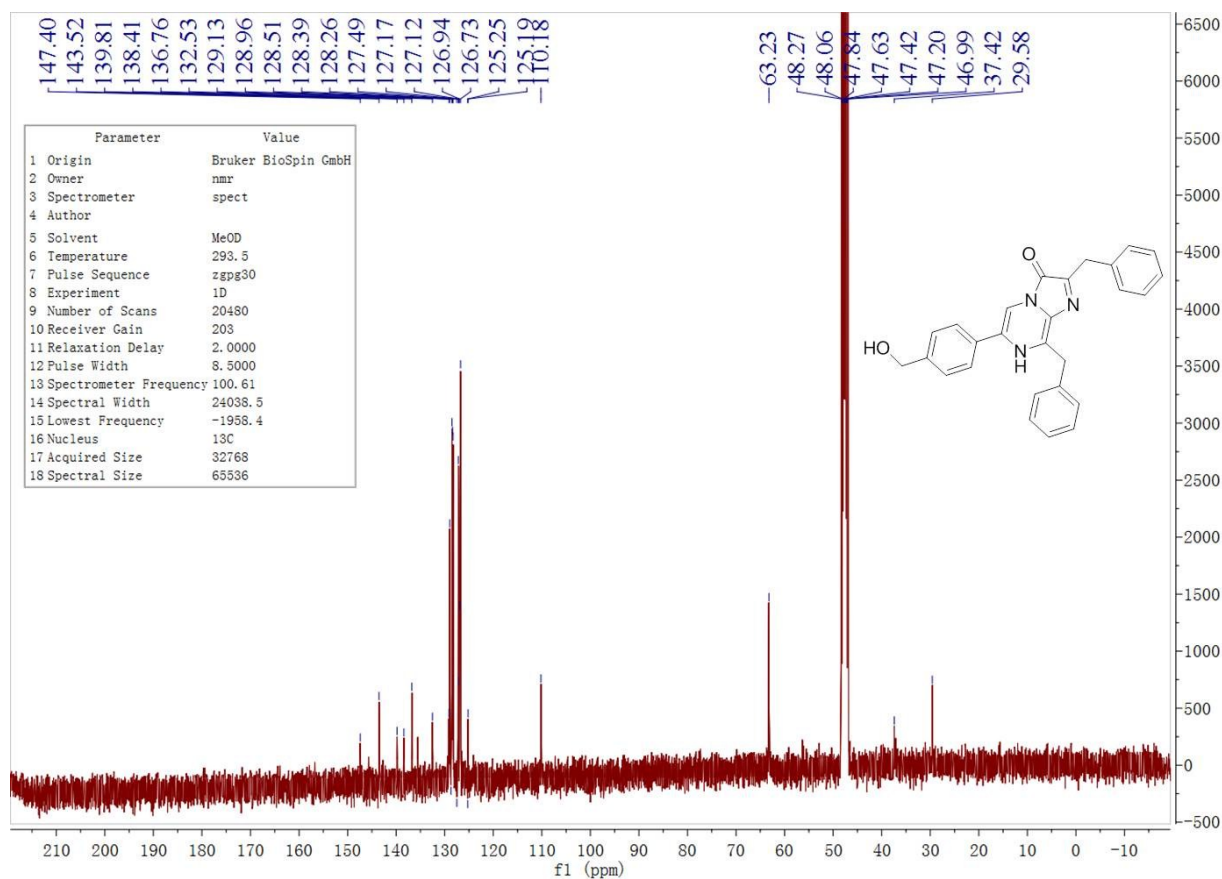
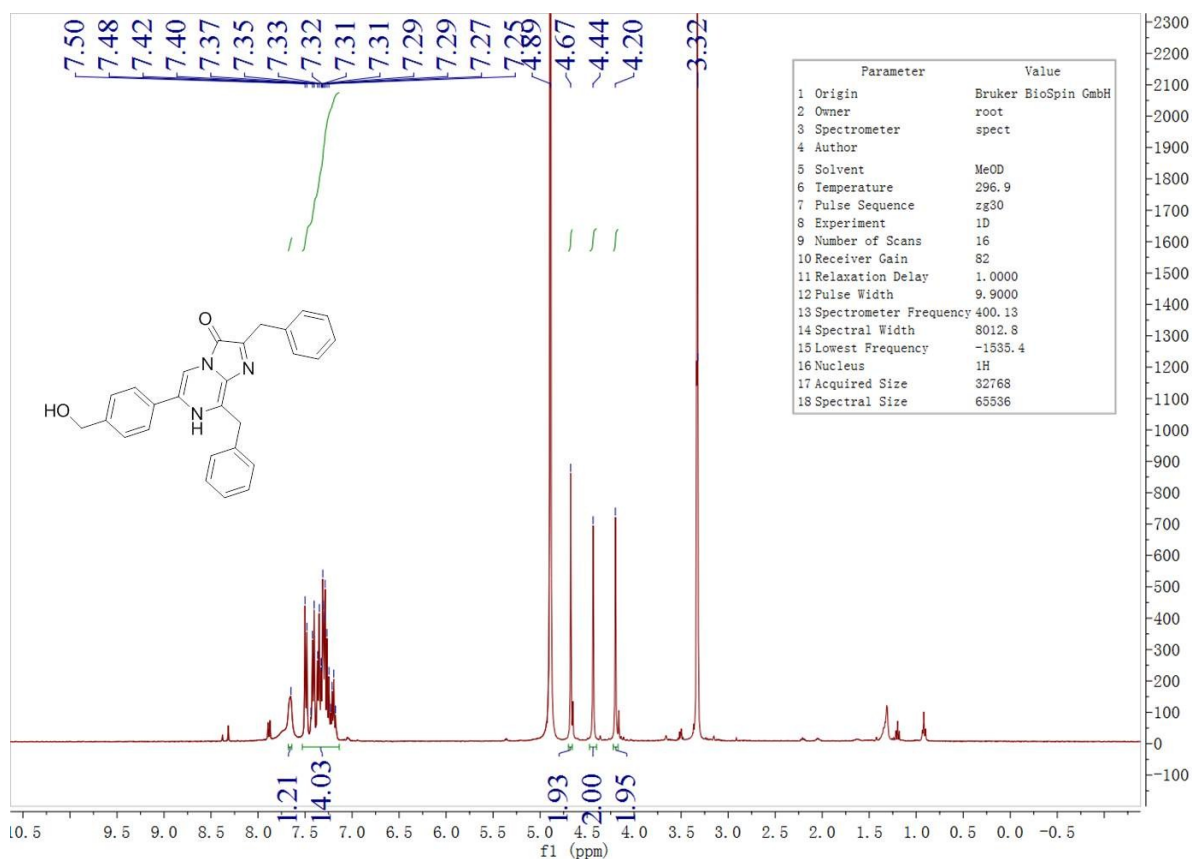
B1



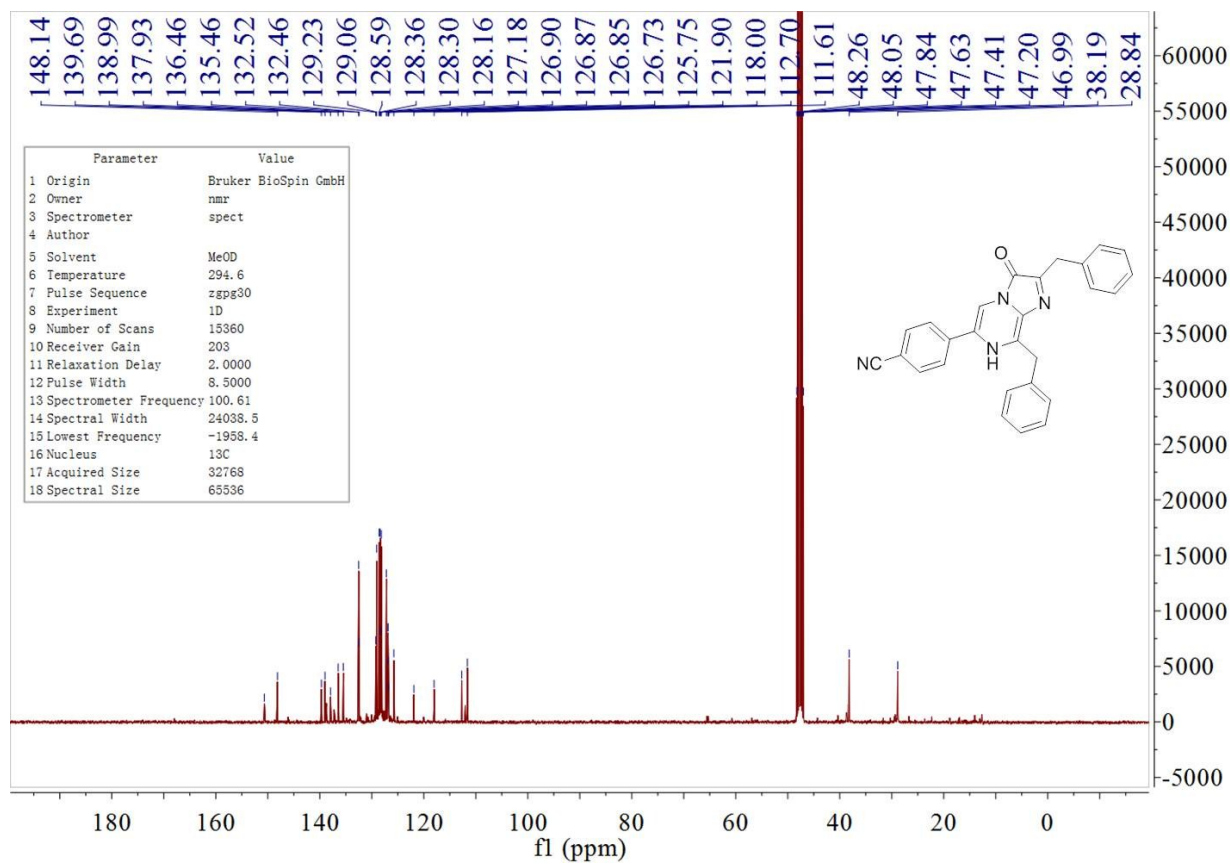
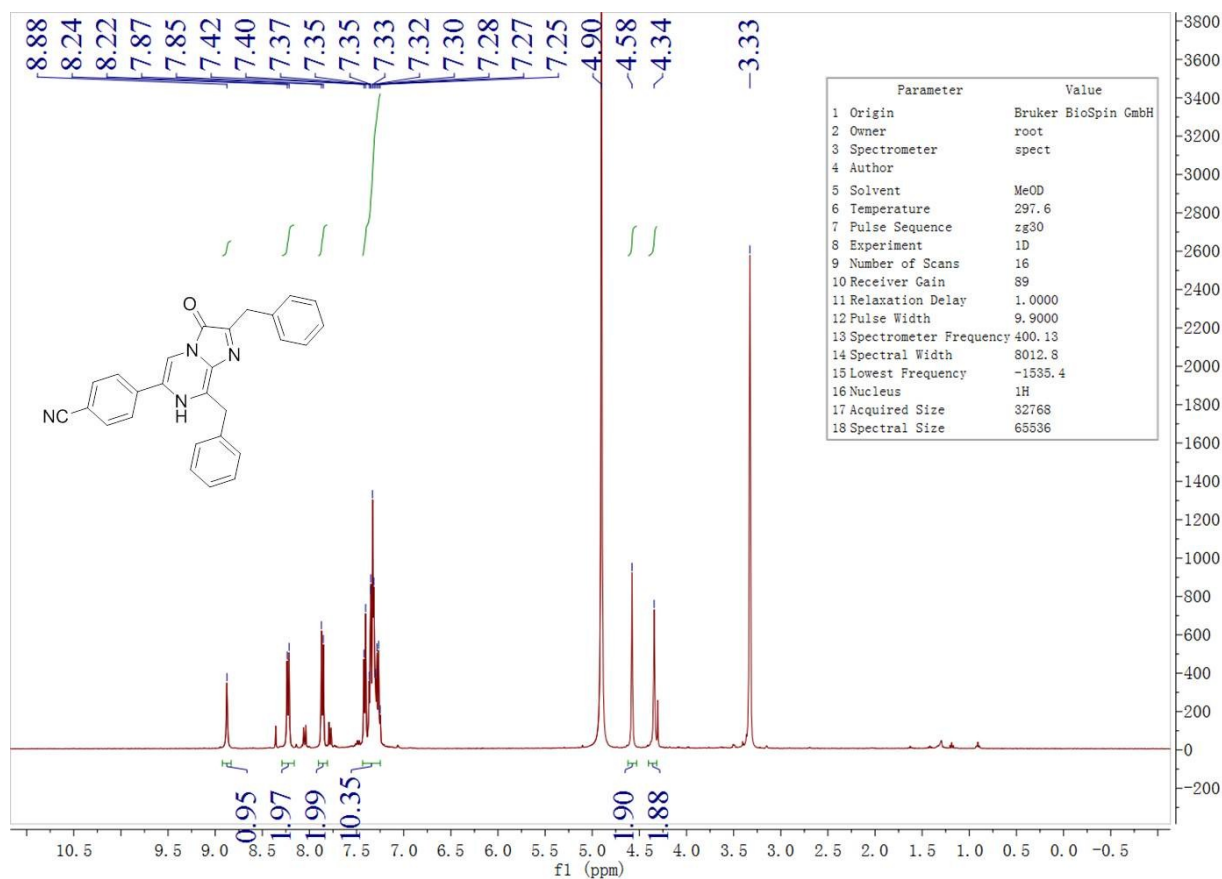
B2



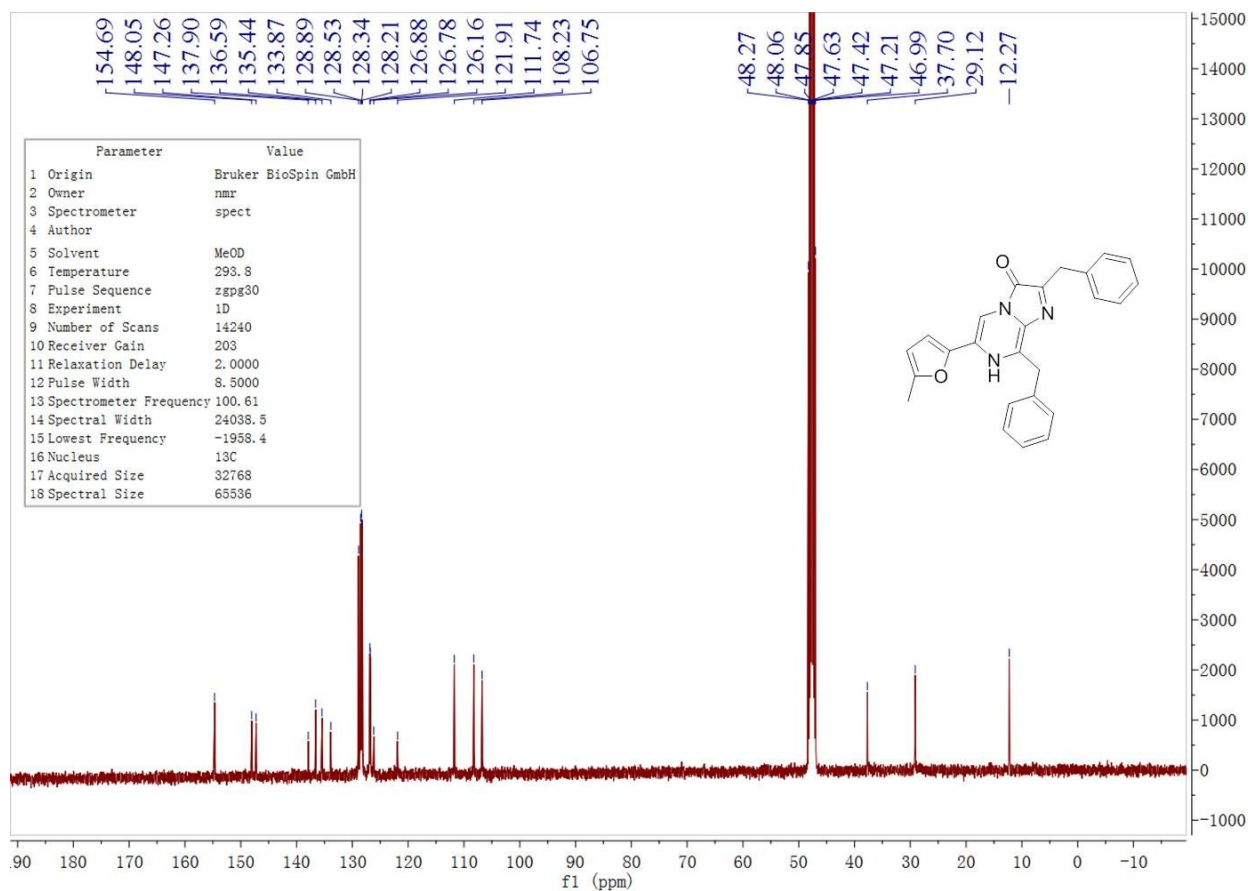
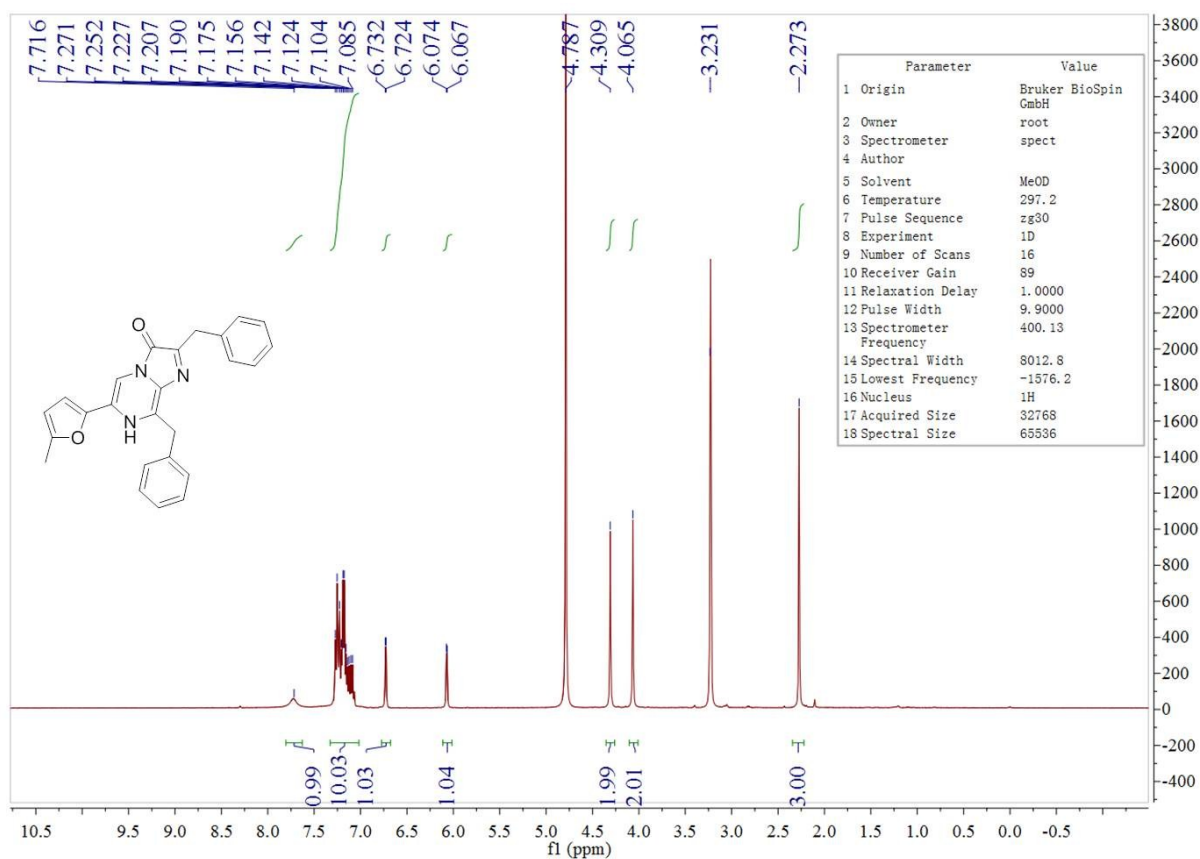
B3



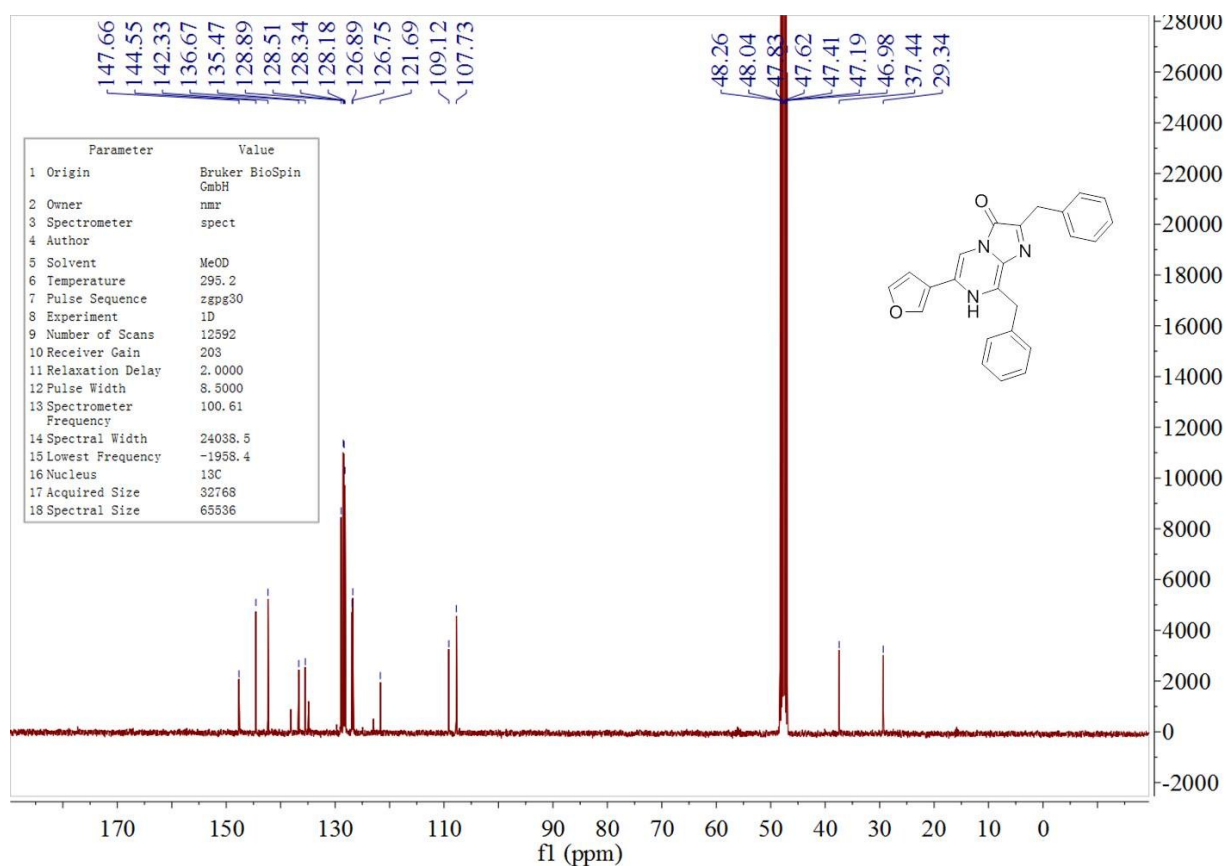
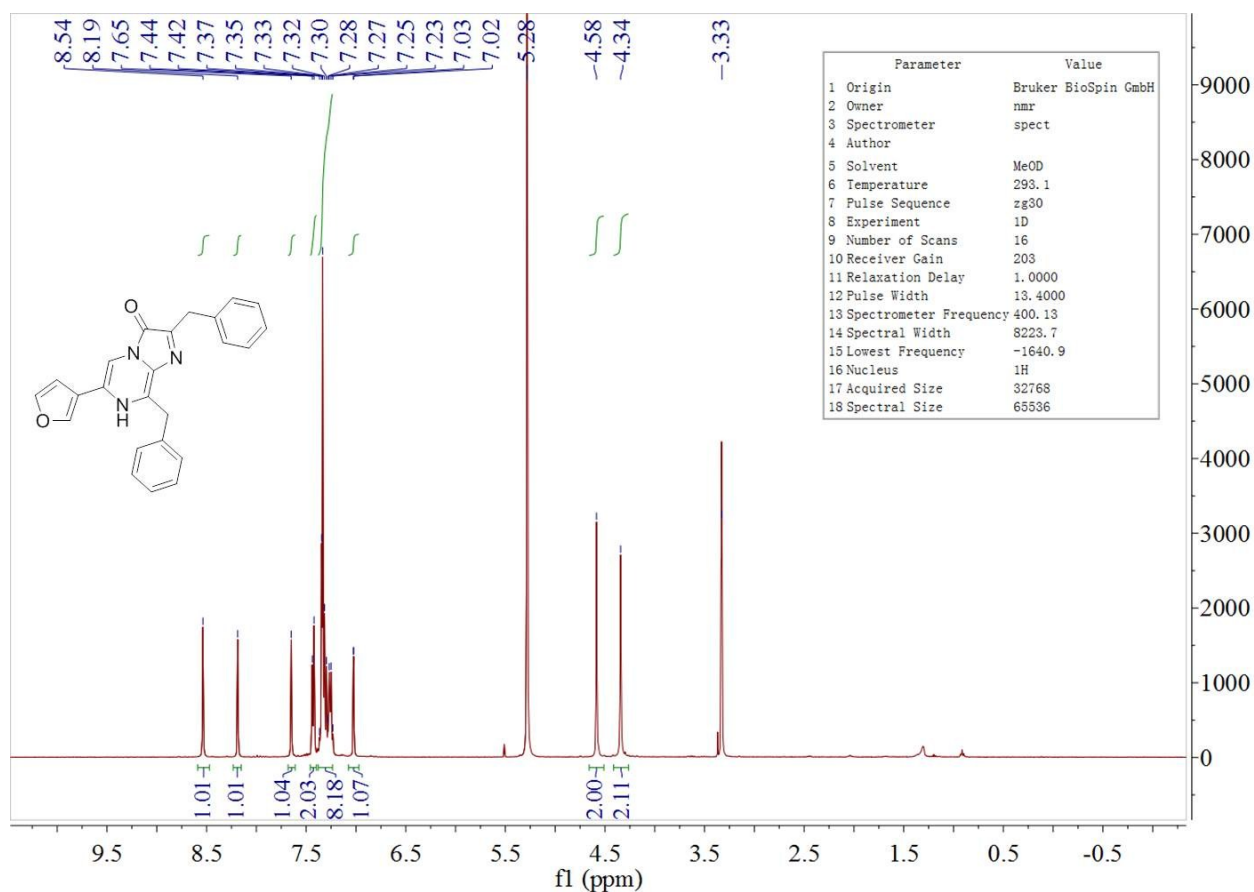
B4



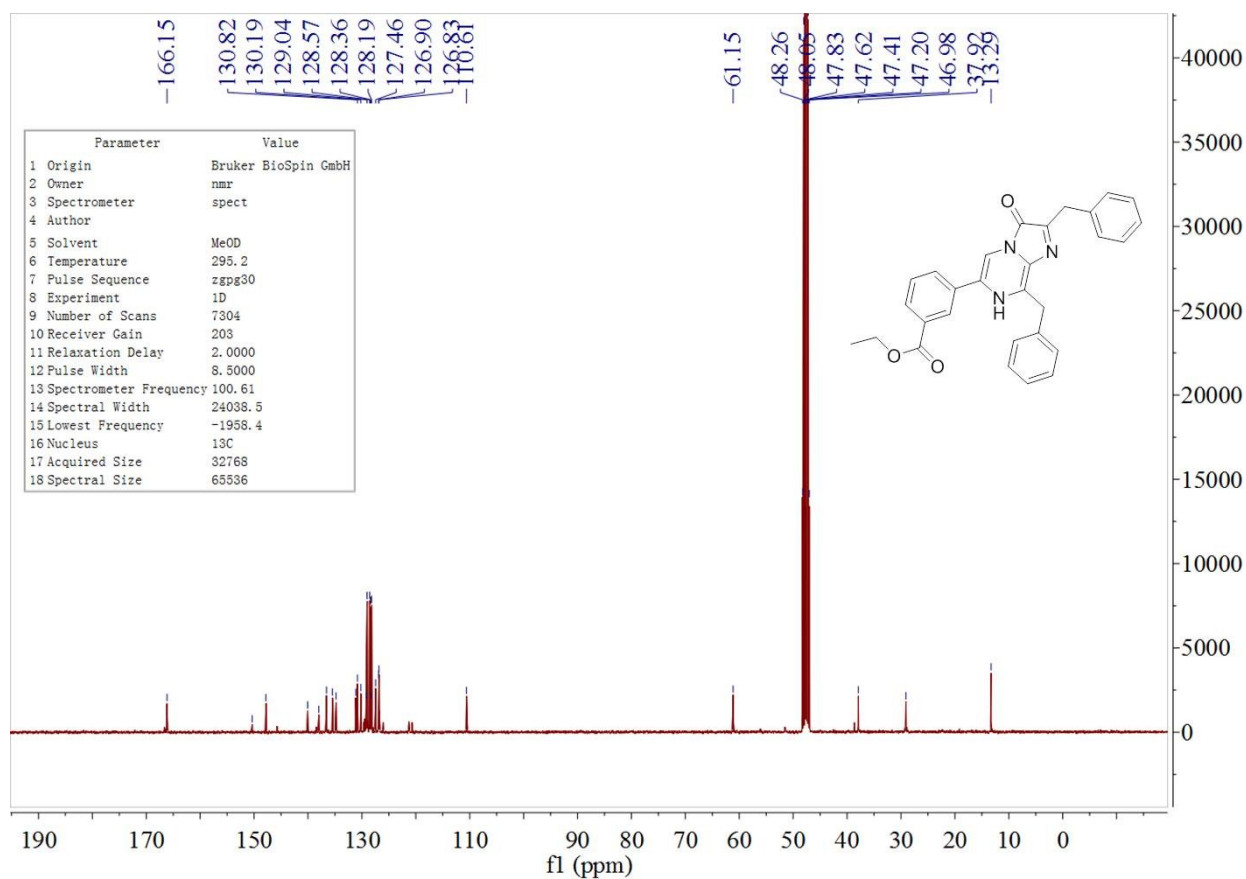
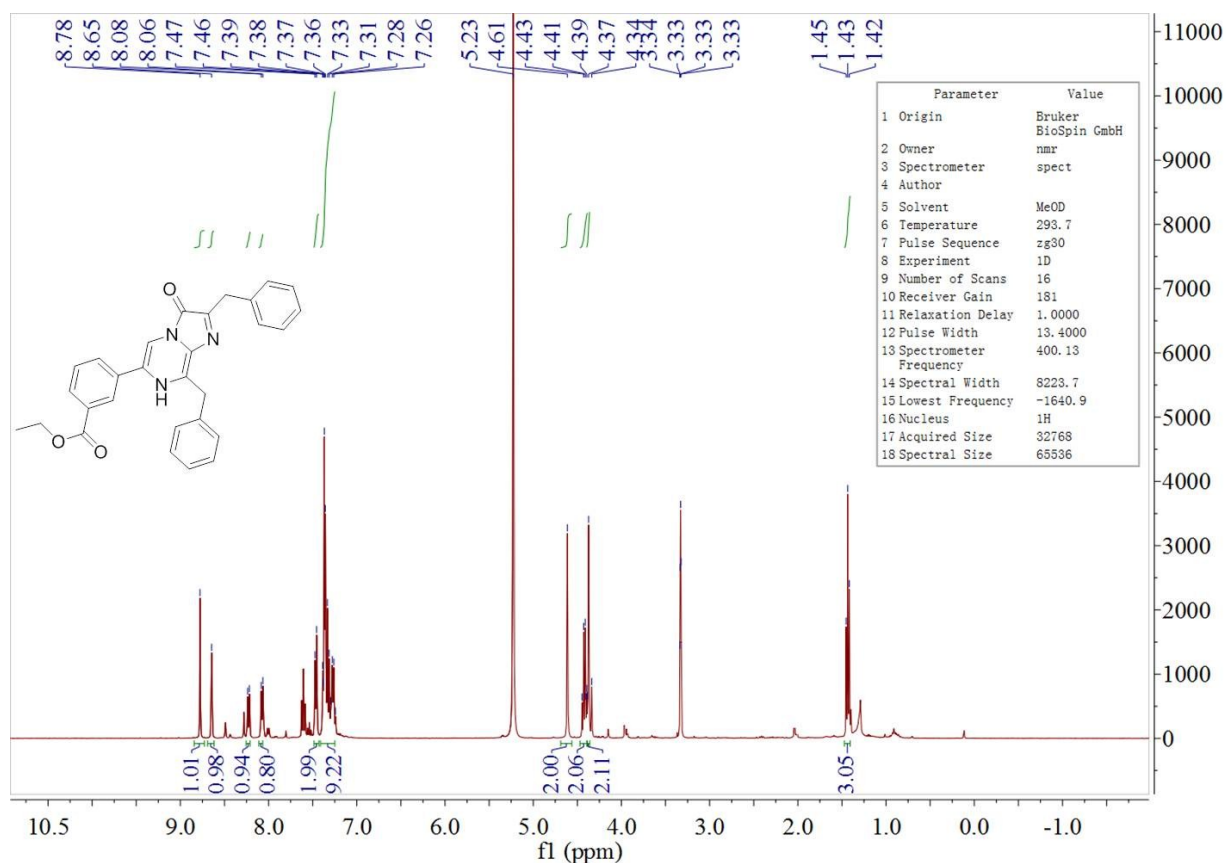
B5



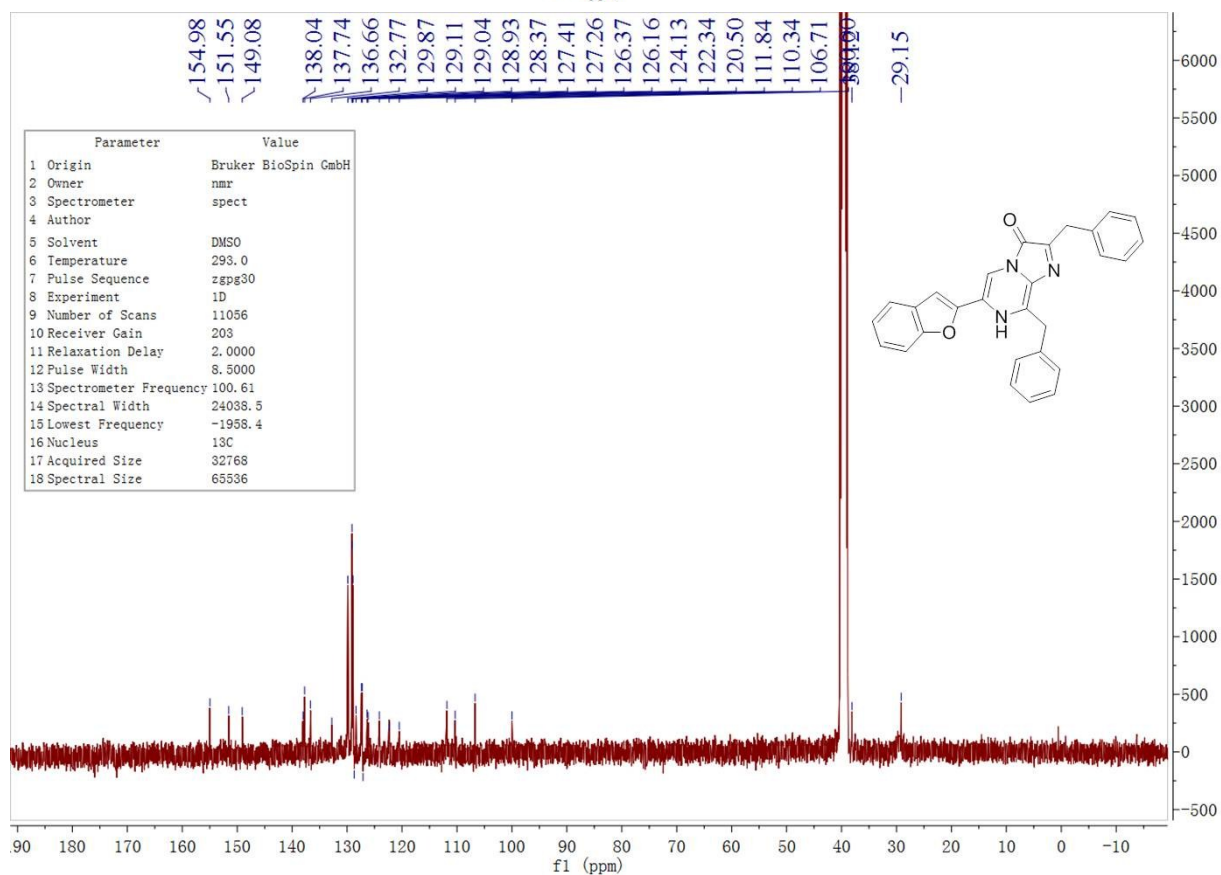
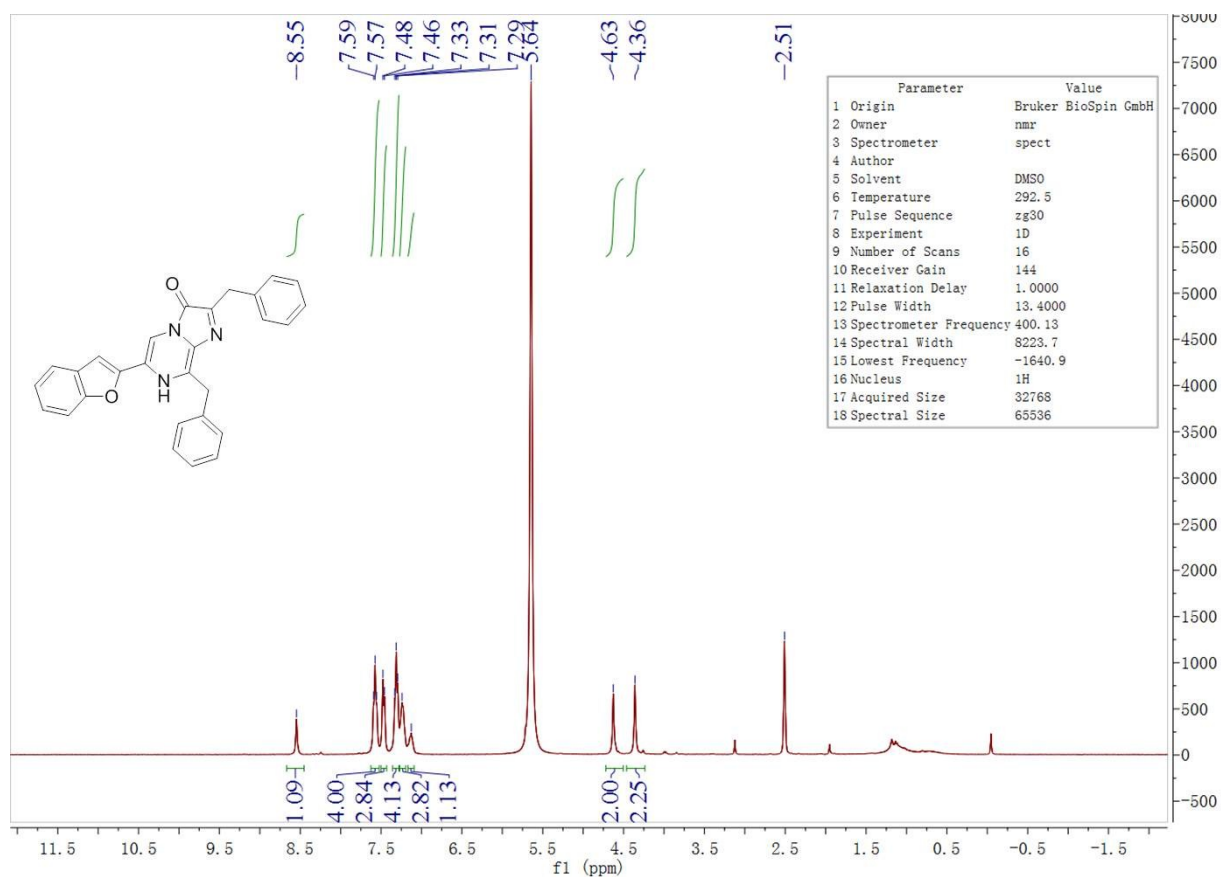
B6



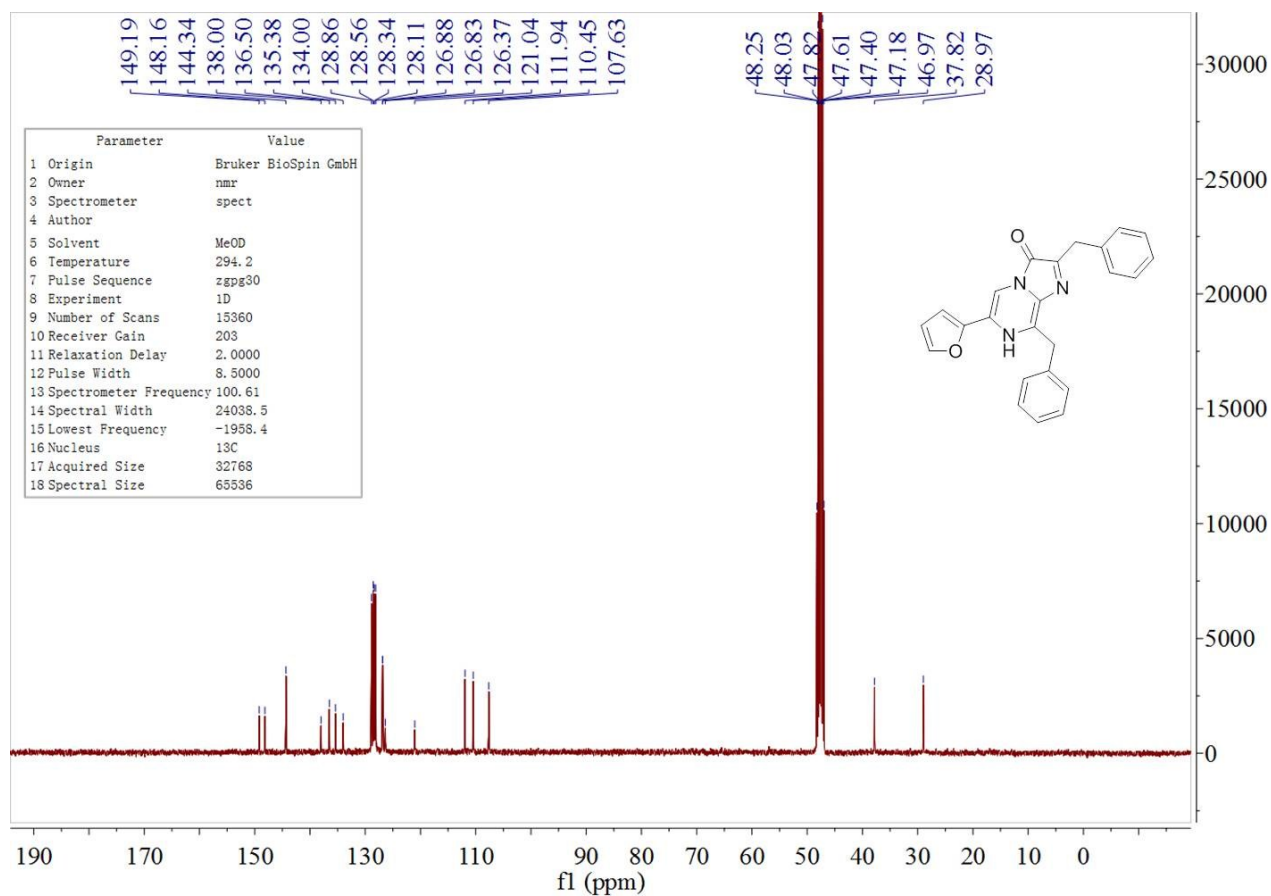
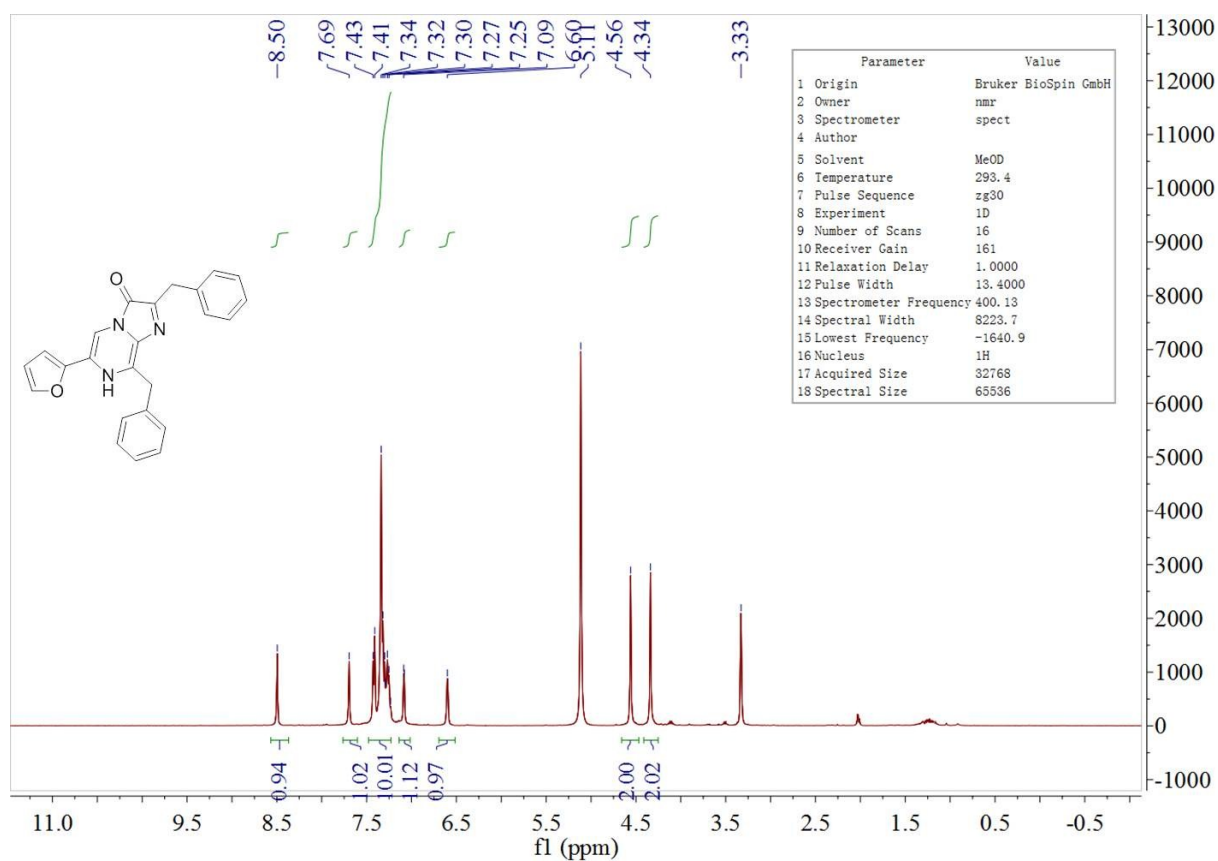
B7



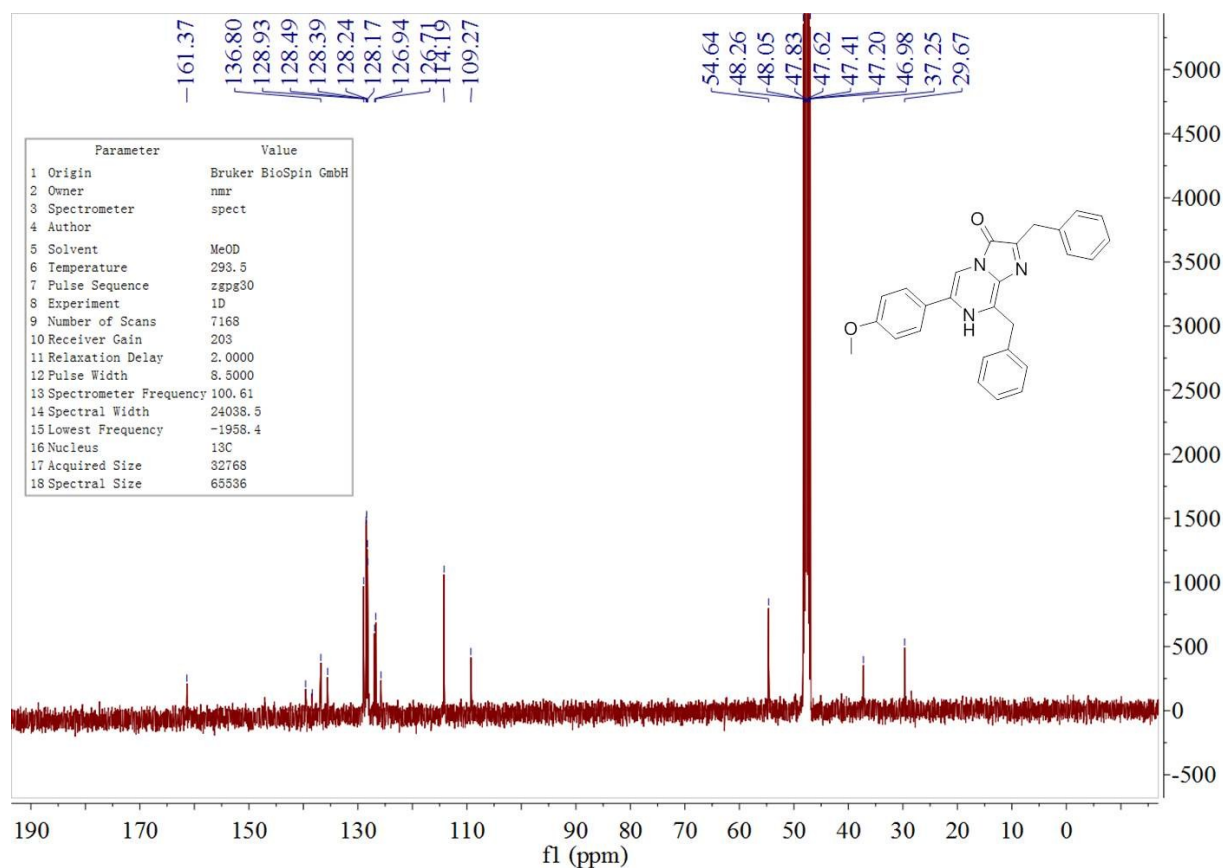
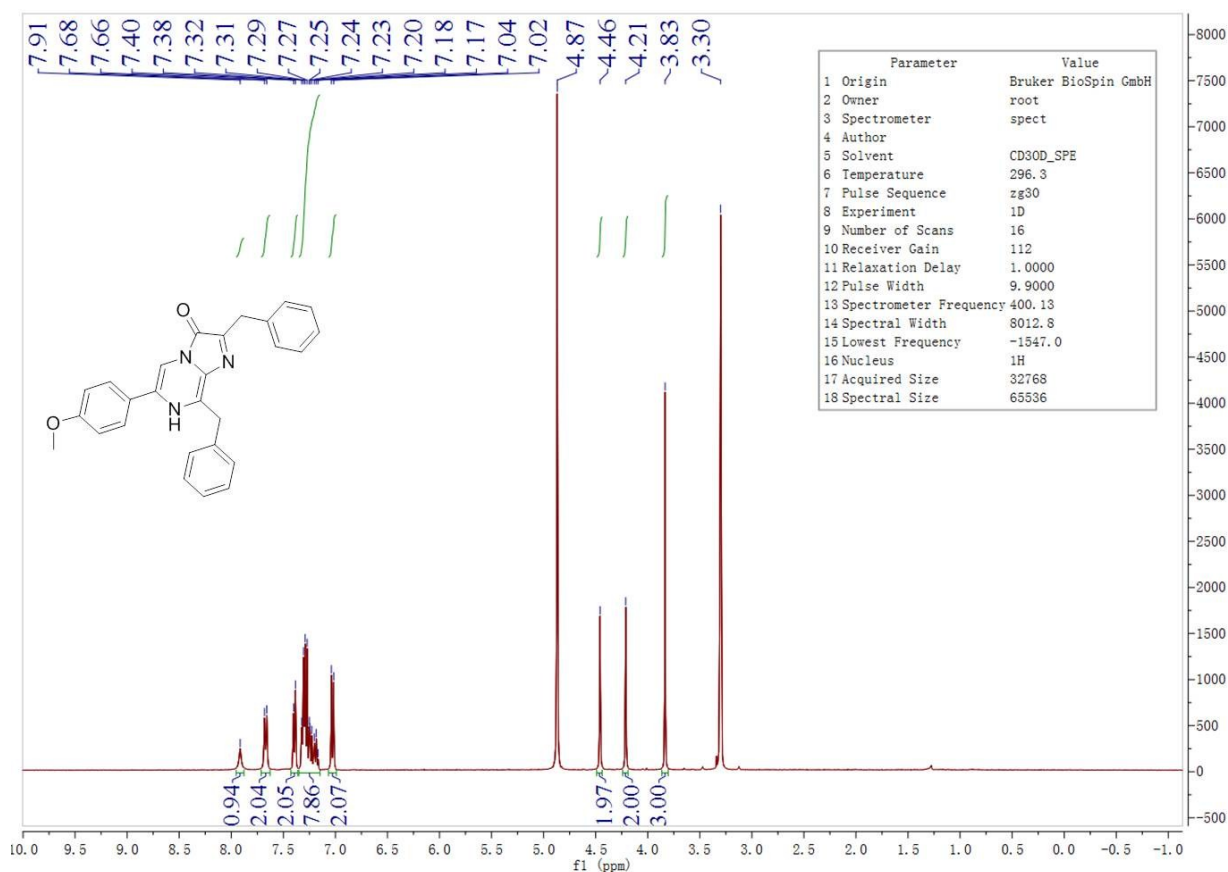
B8



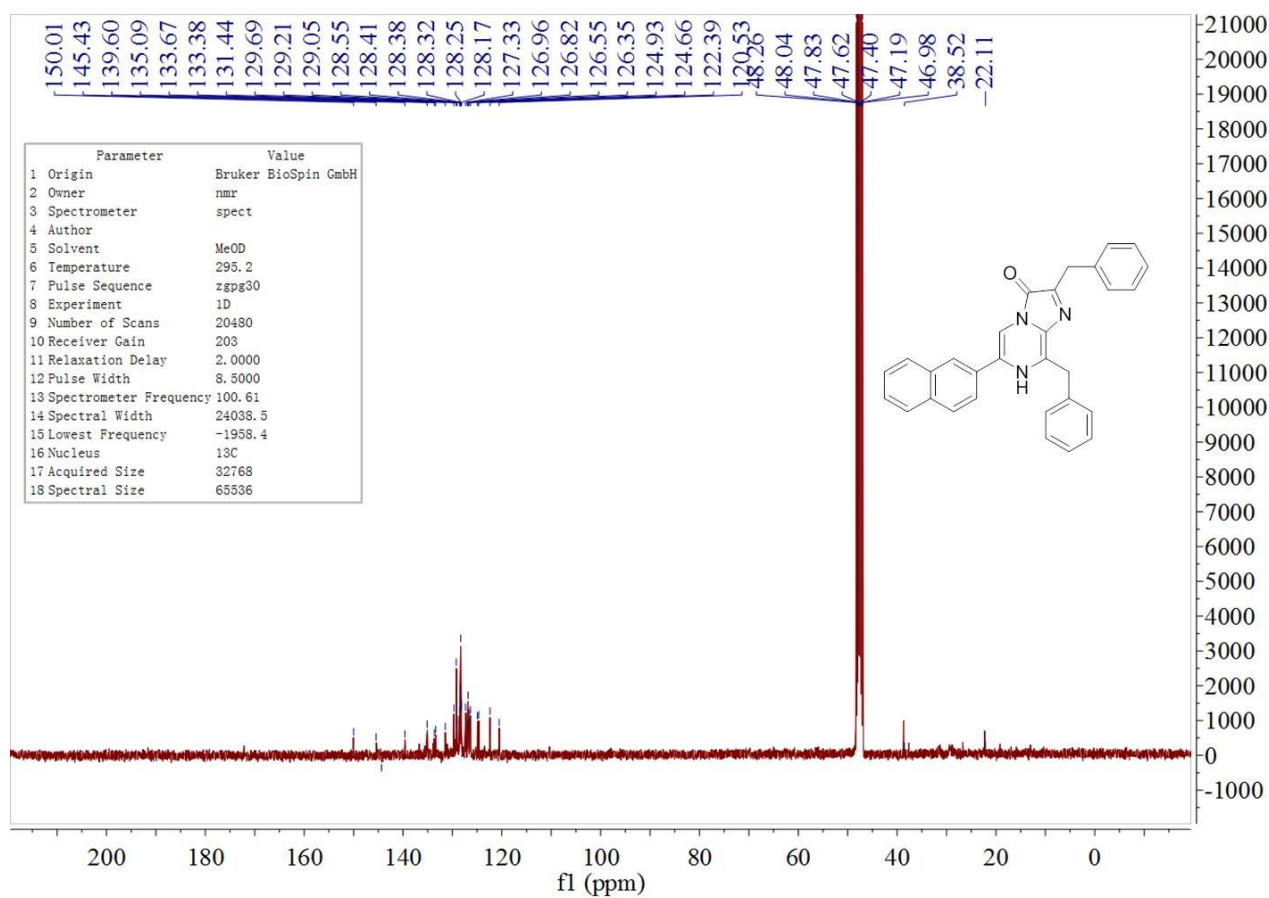
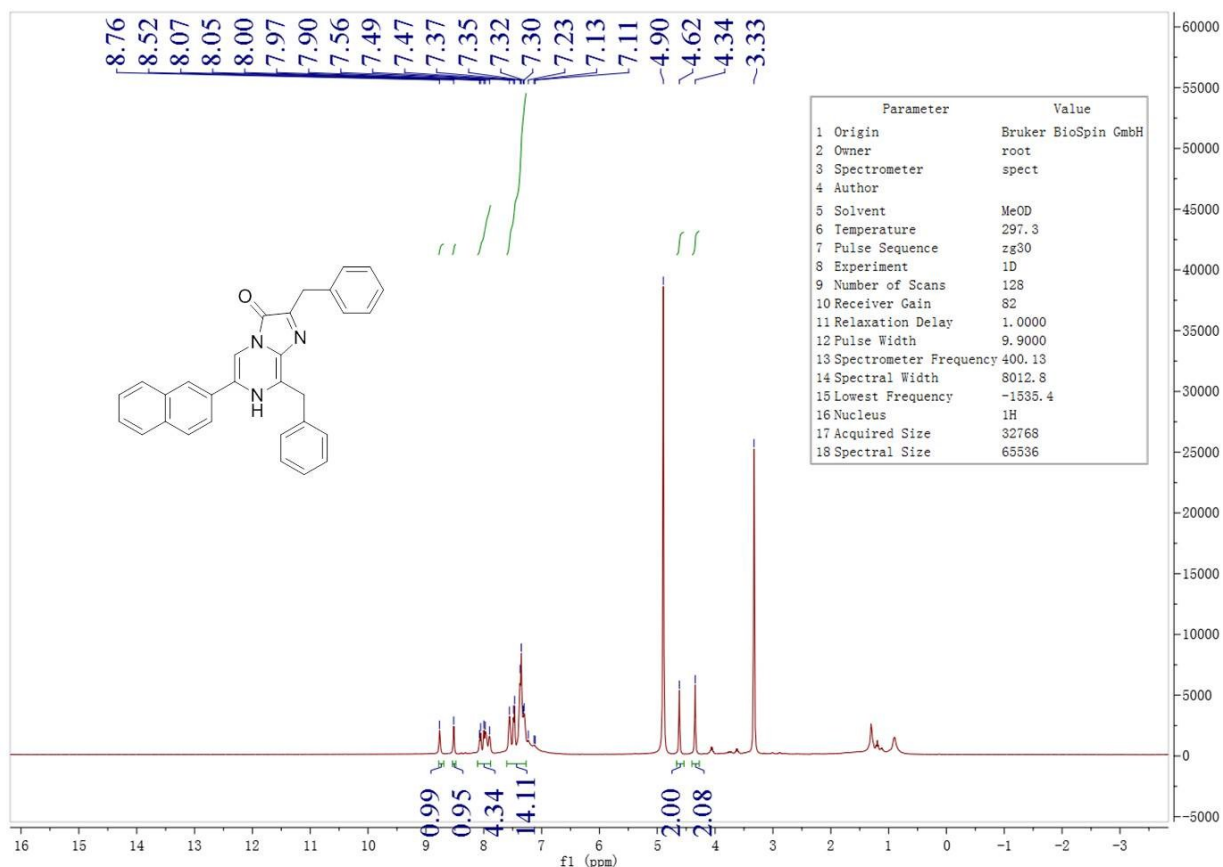
B9



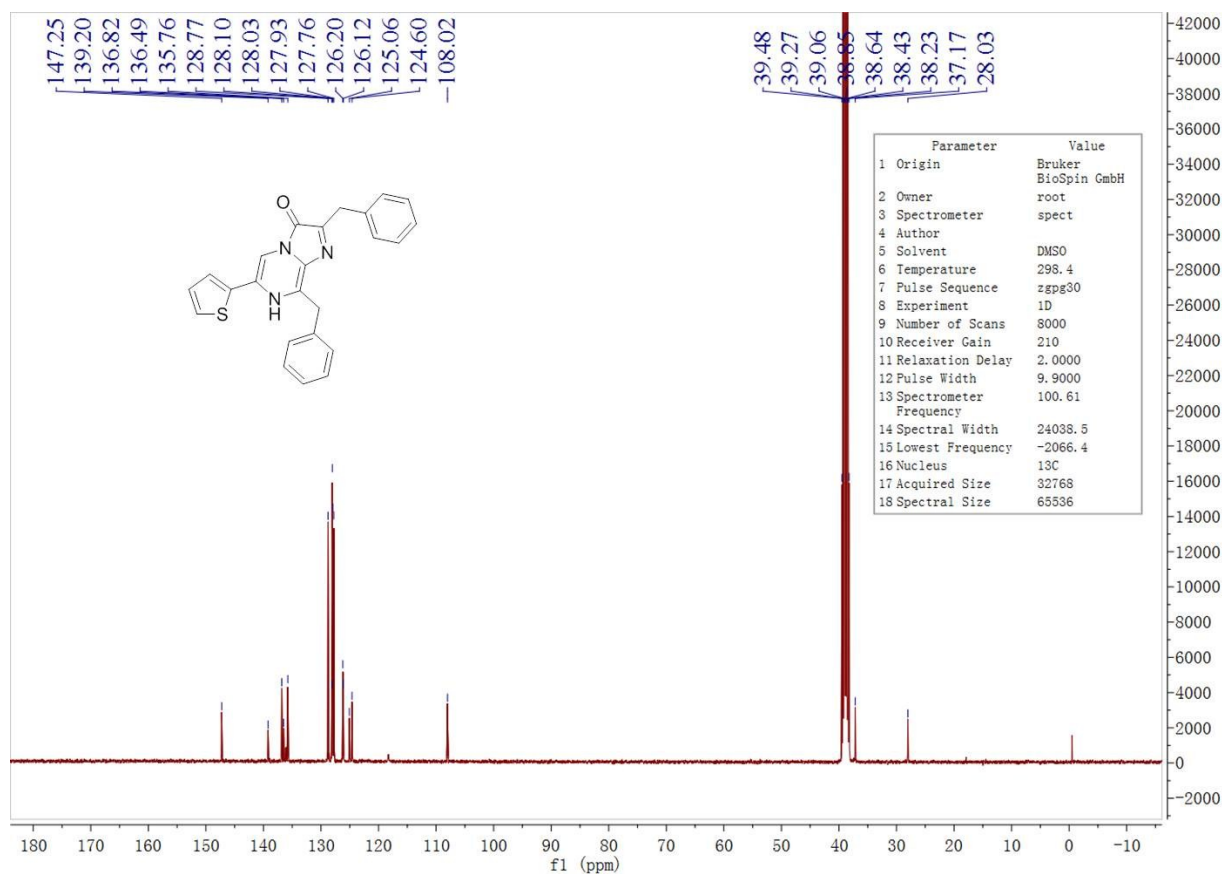
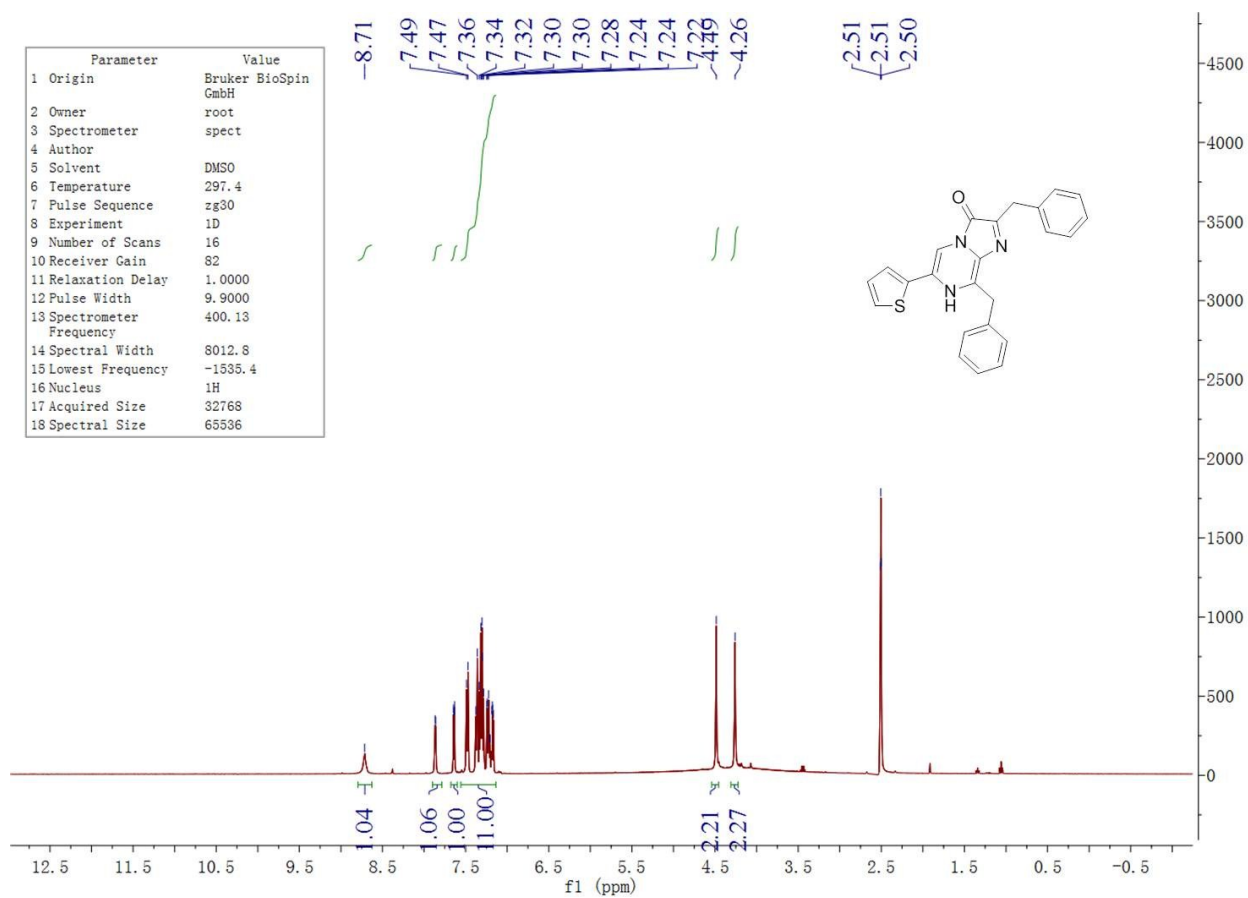
B10



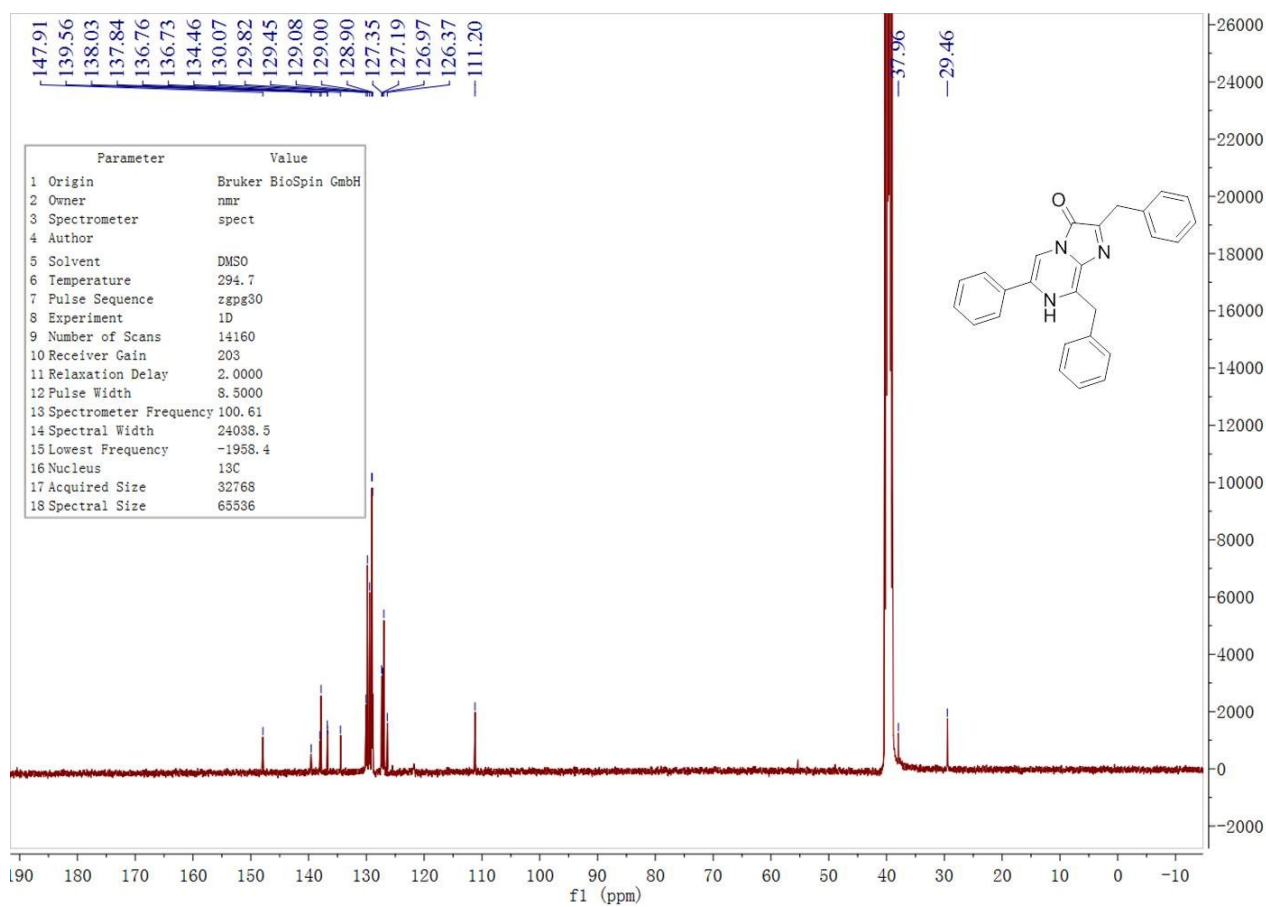
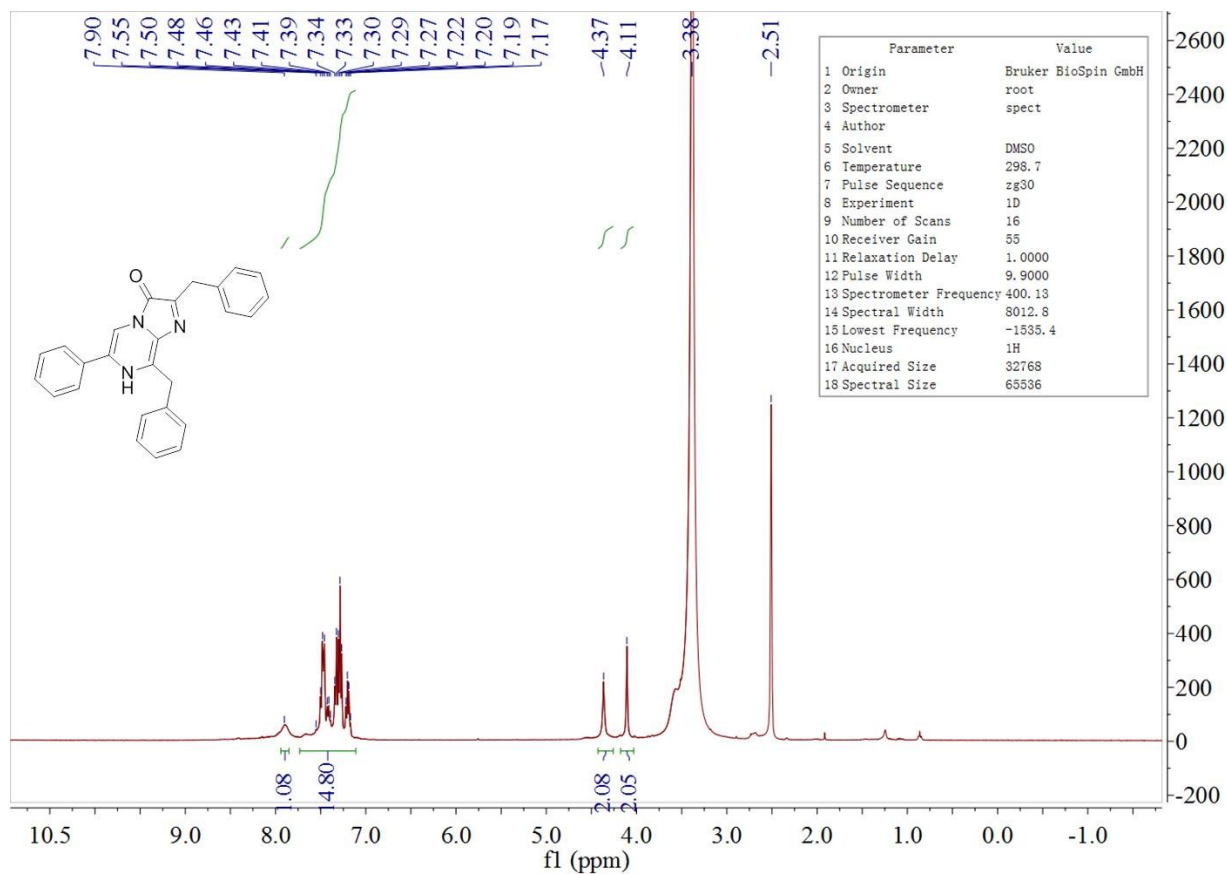
B11



B12

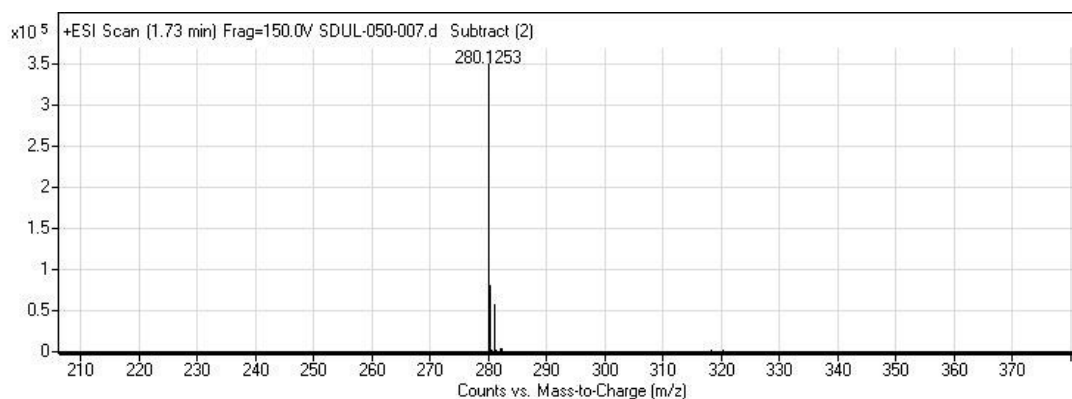


B13

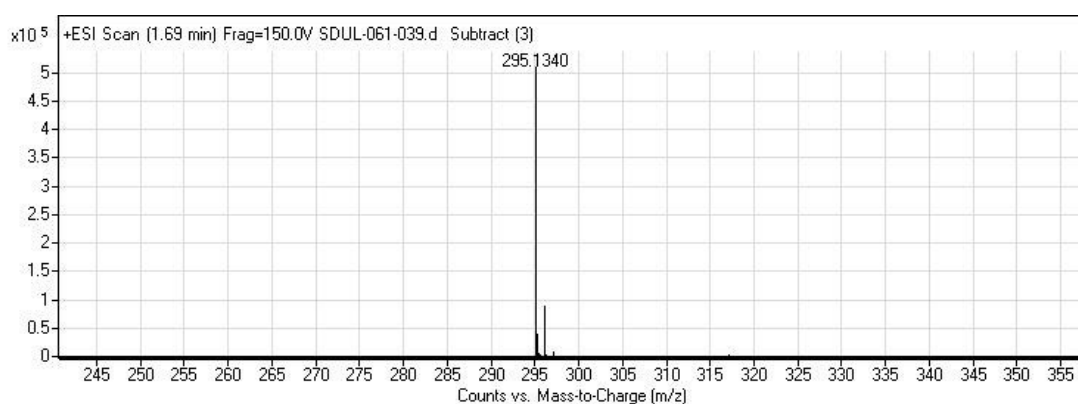


HRMS spectra

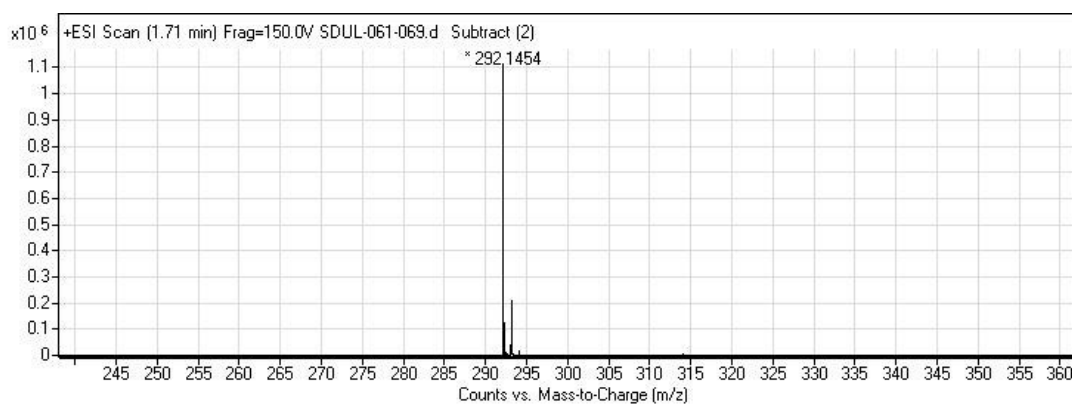
2-1



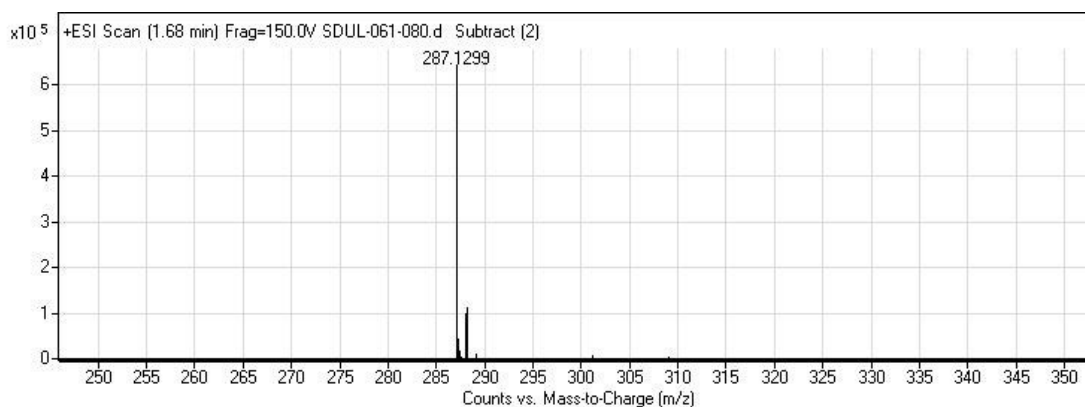
2-2



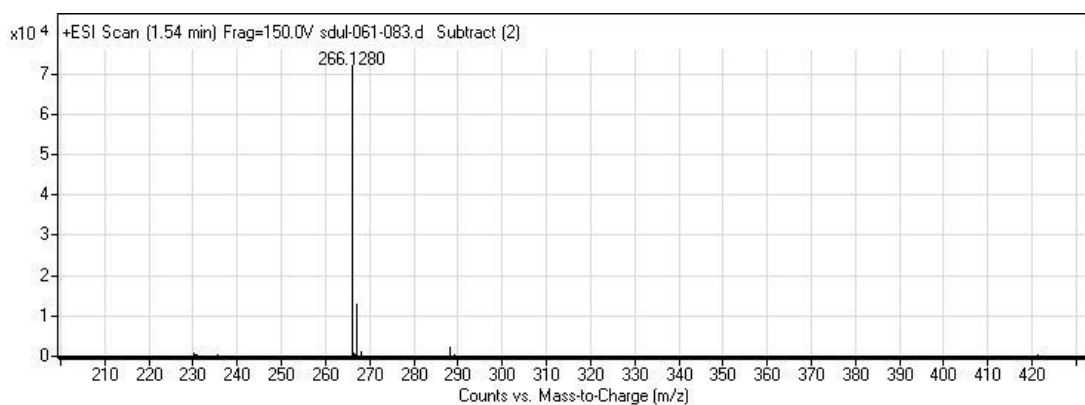
2-3



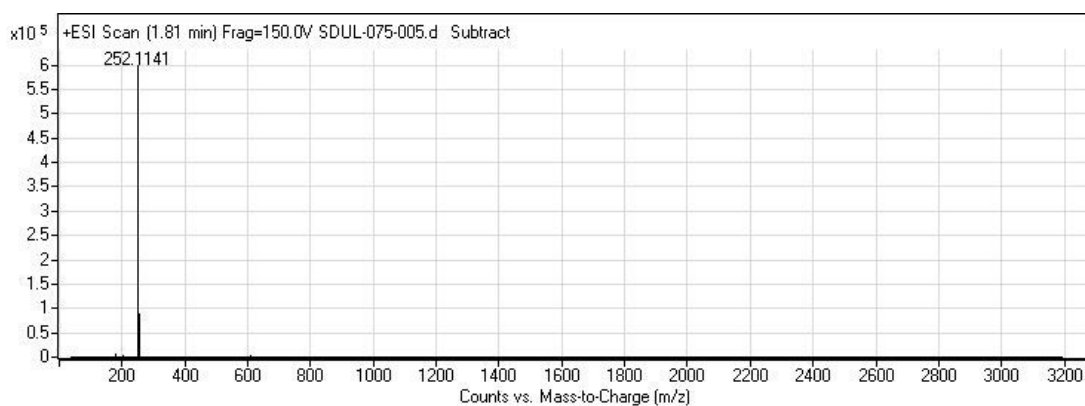
2-4



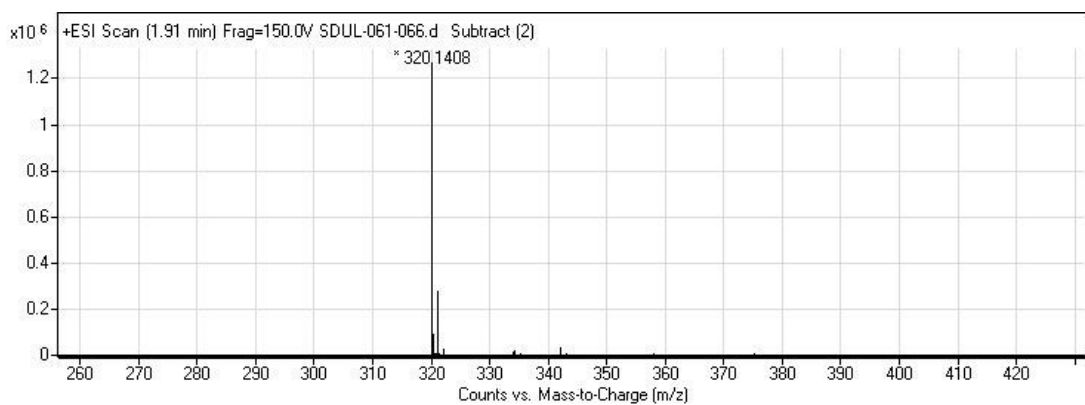
2-5



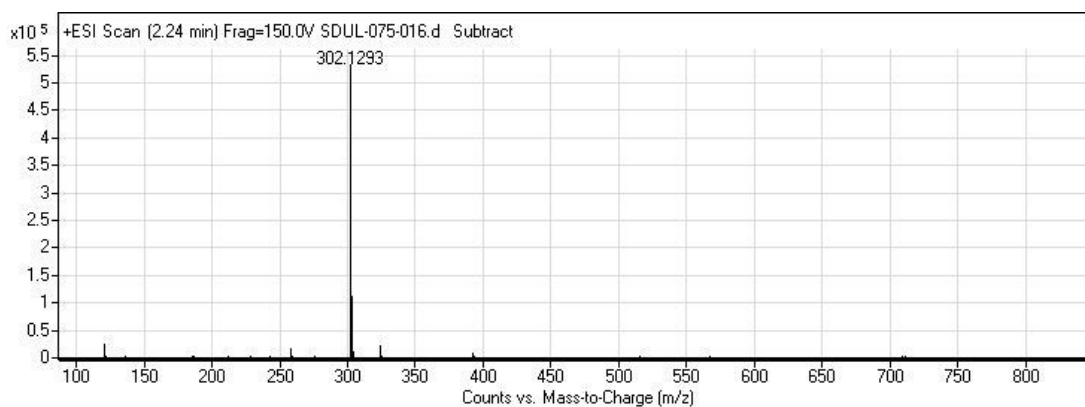
2-6



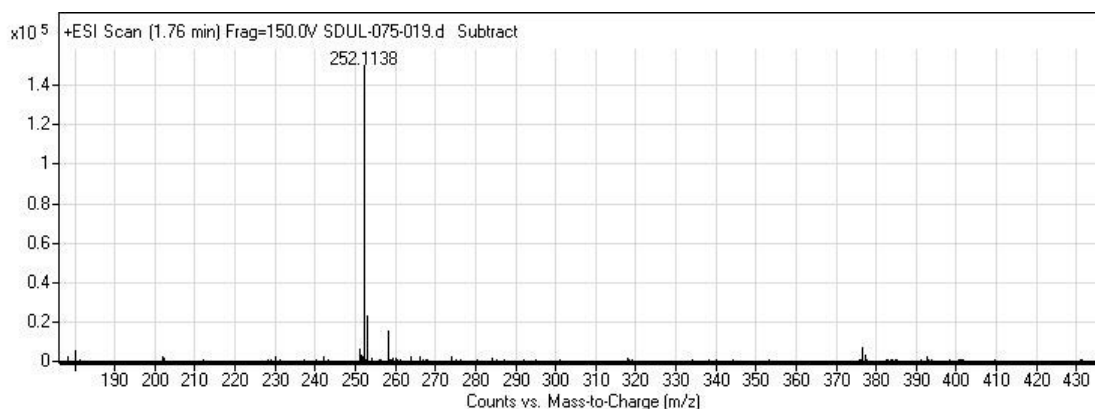
2-7



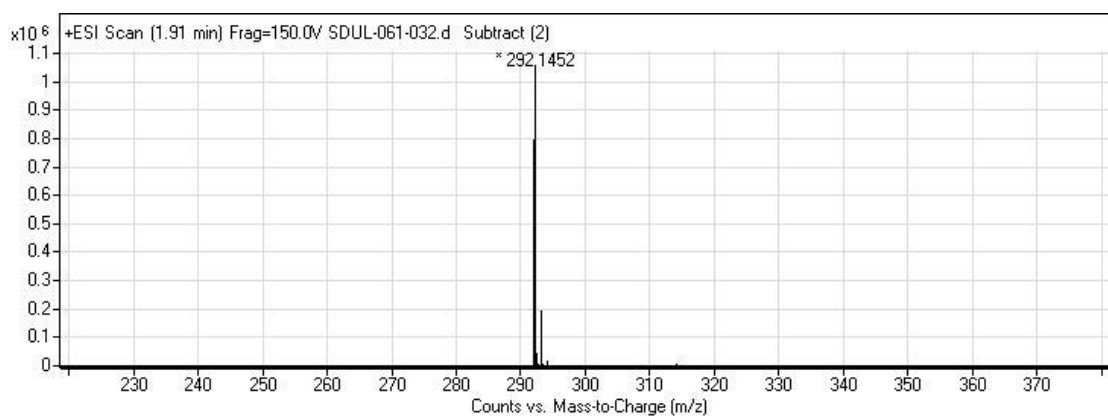
2-8



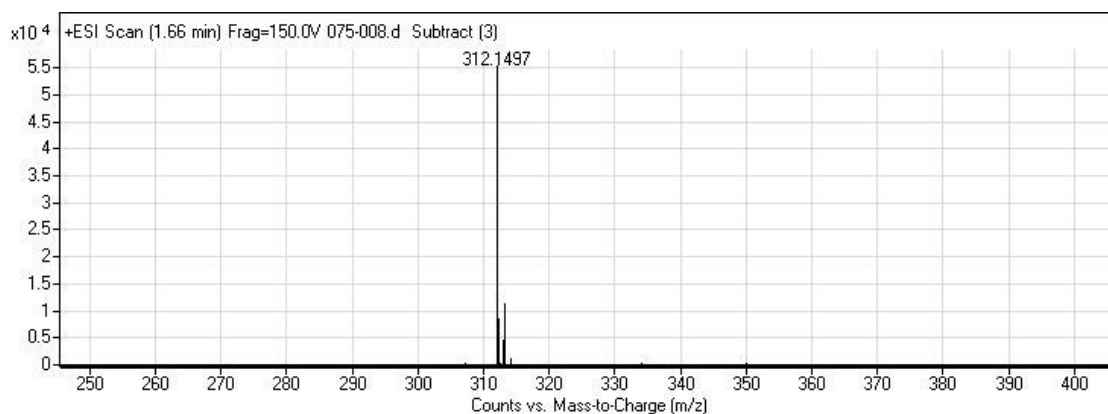
2-9



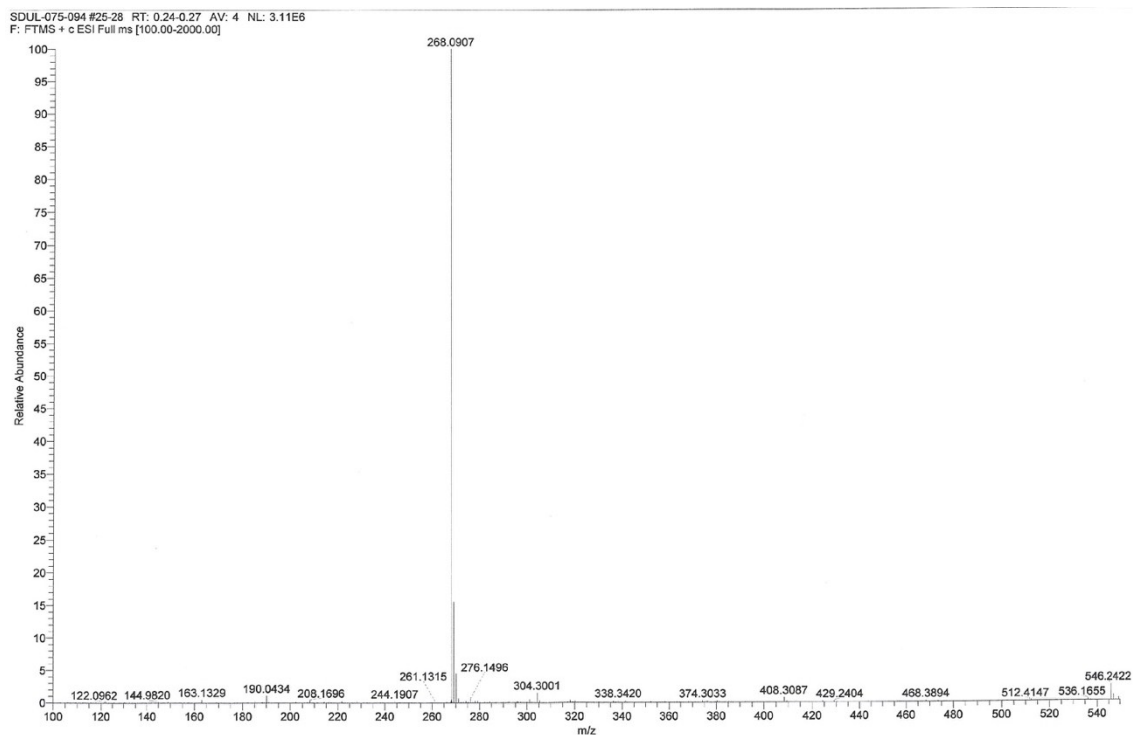
2-10



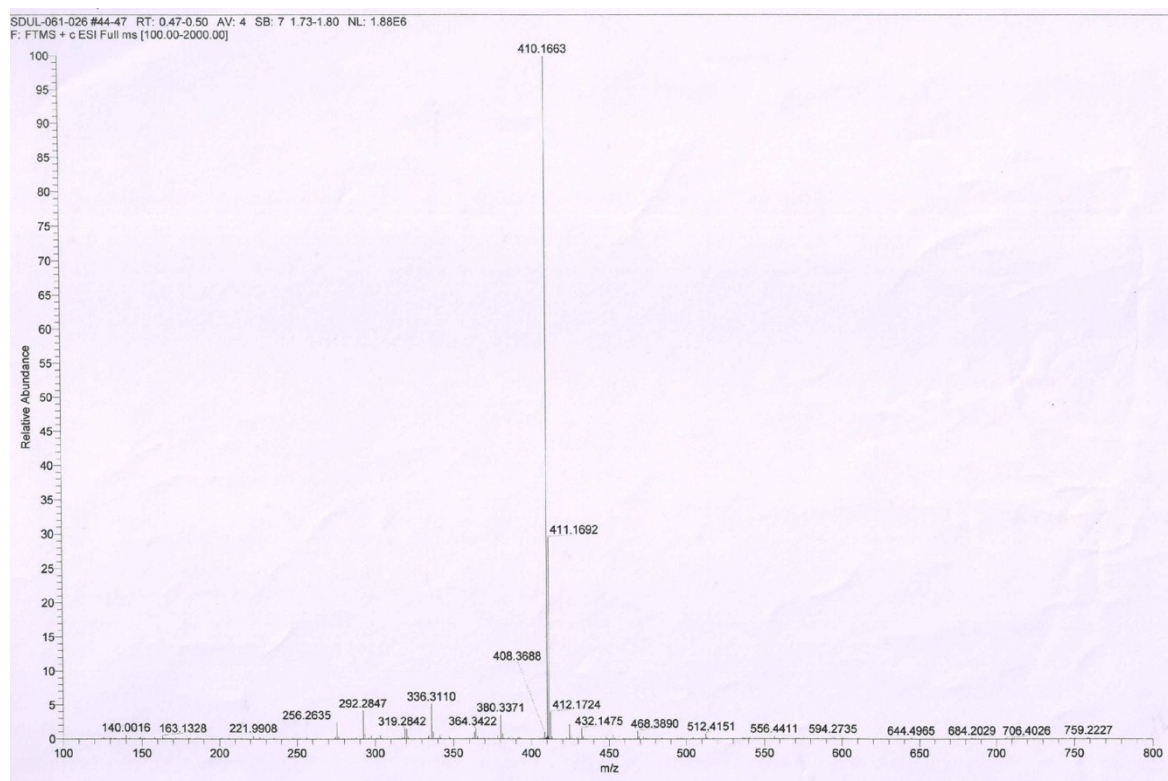
2-11



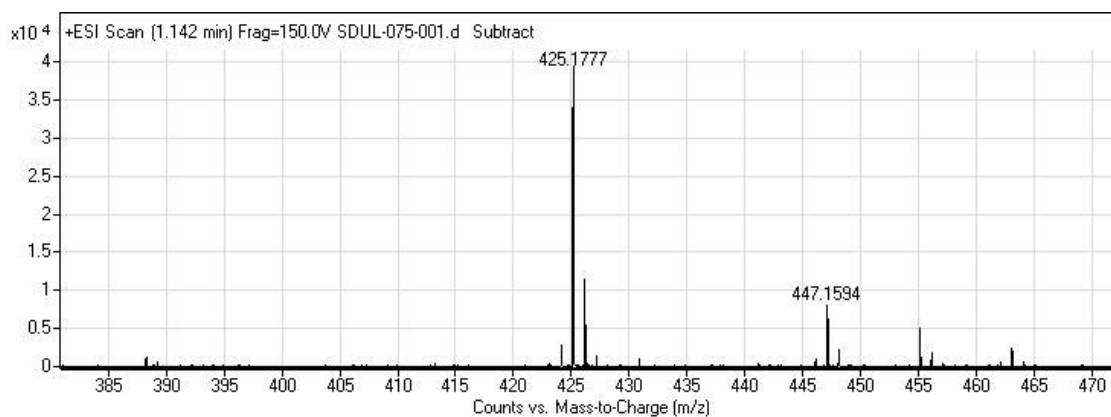
2-12



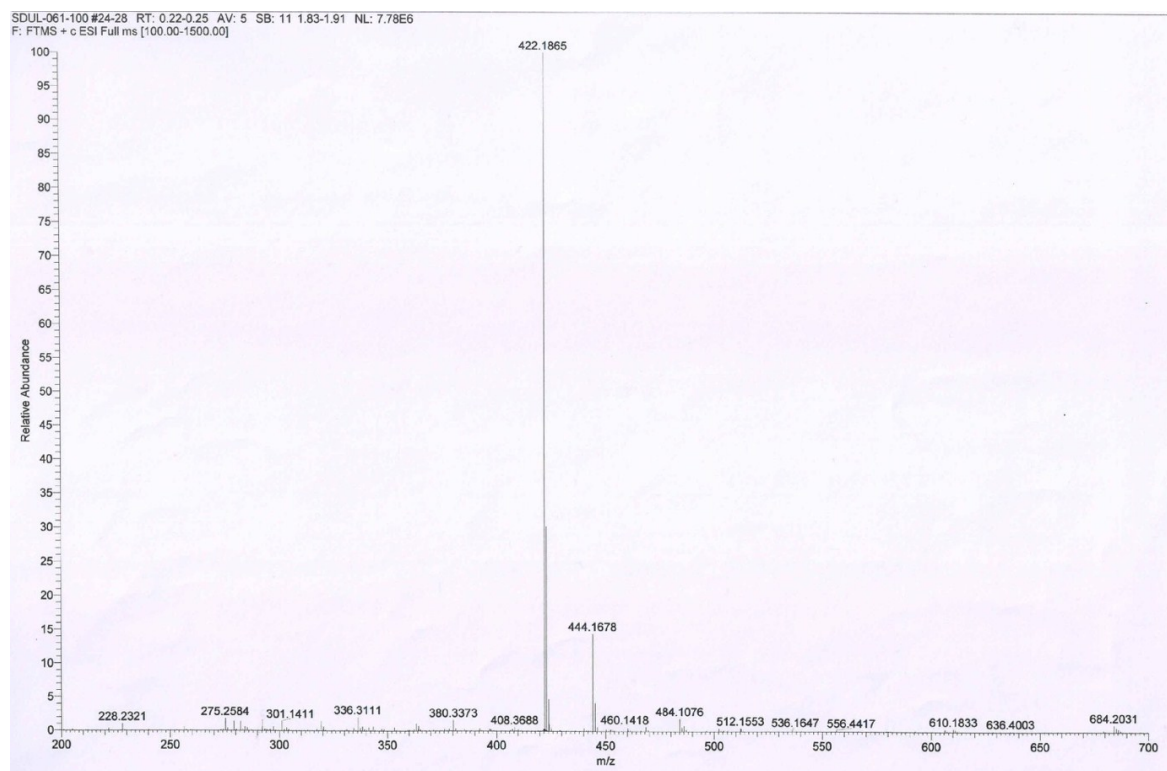
B1



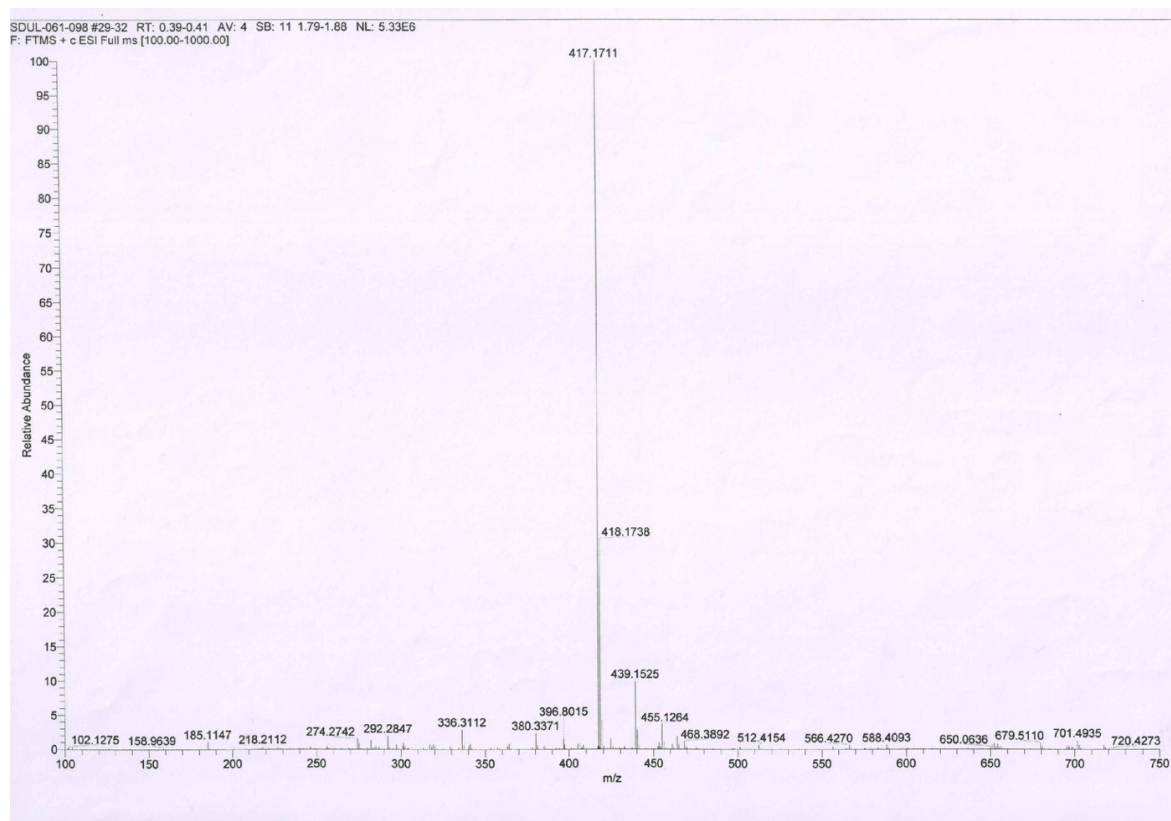
B2



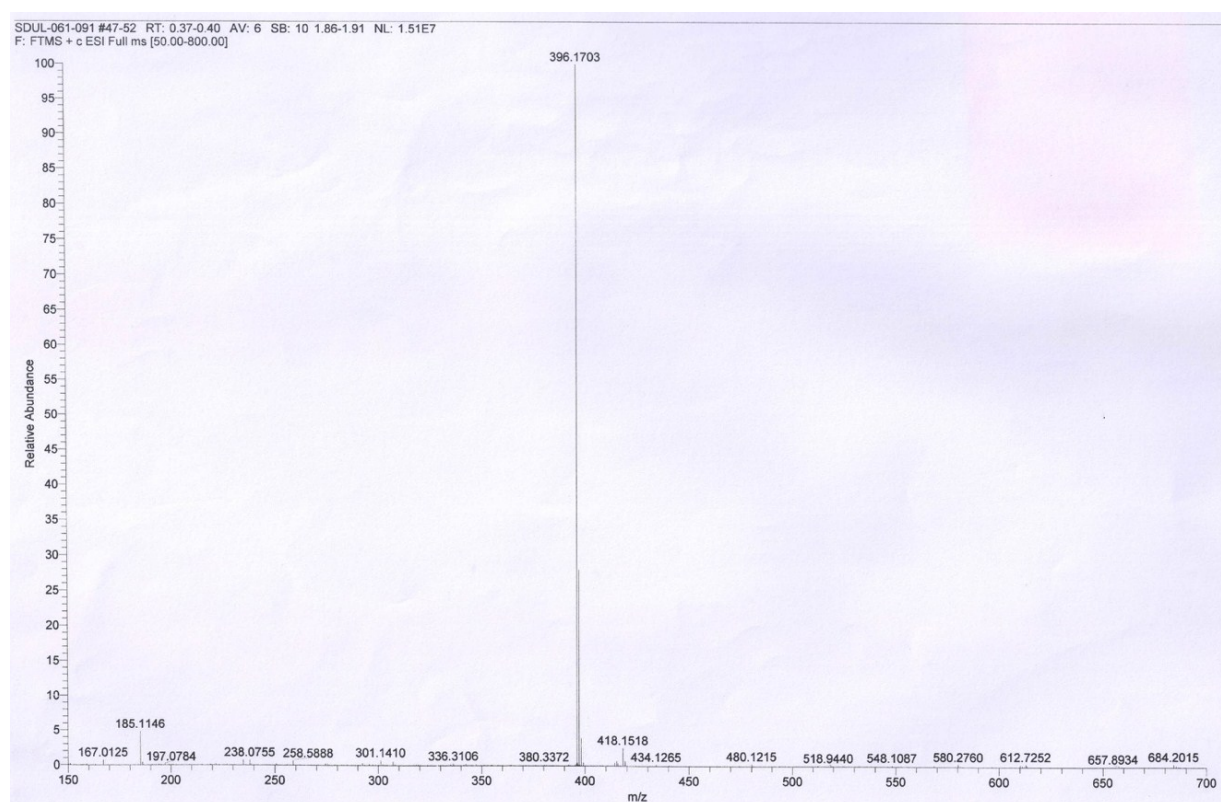
B3



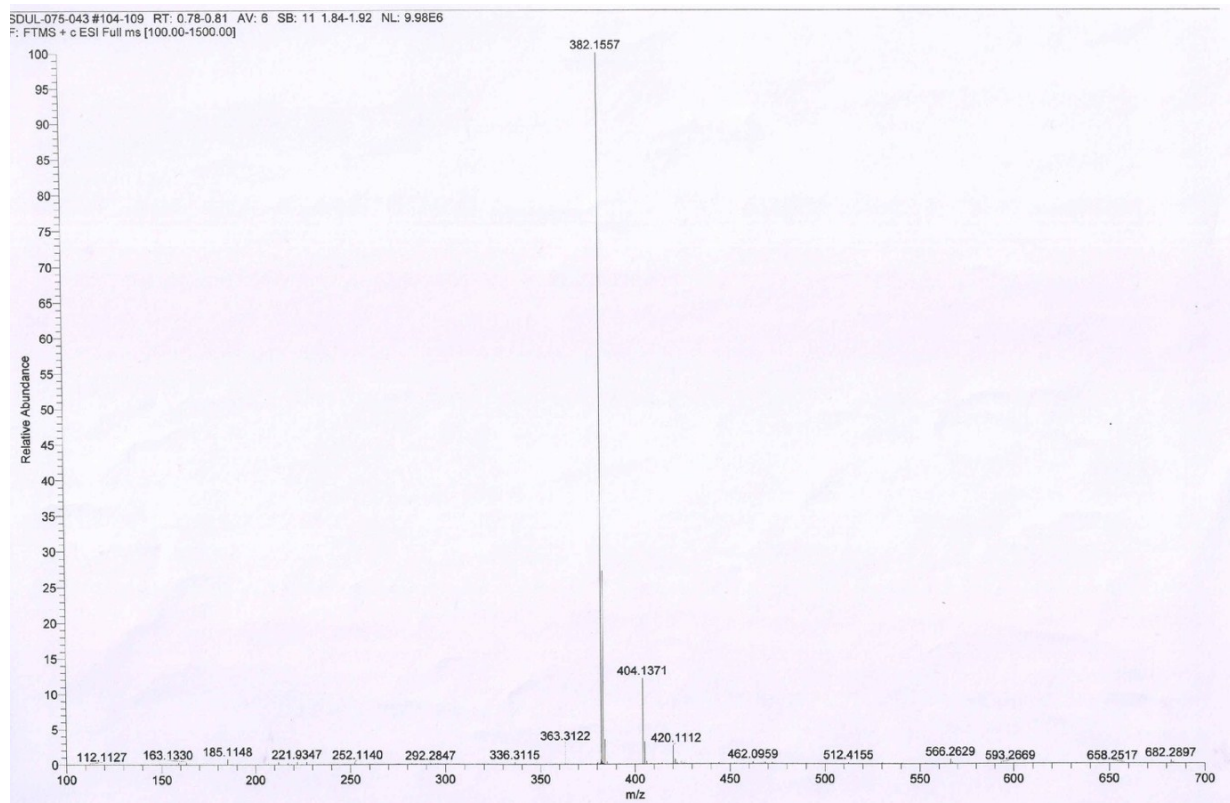
B4



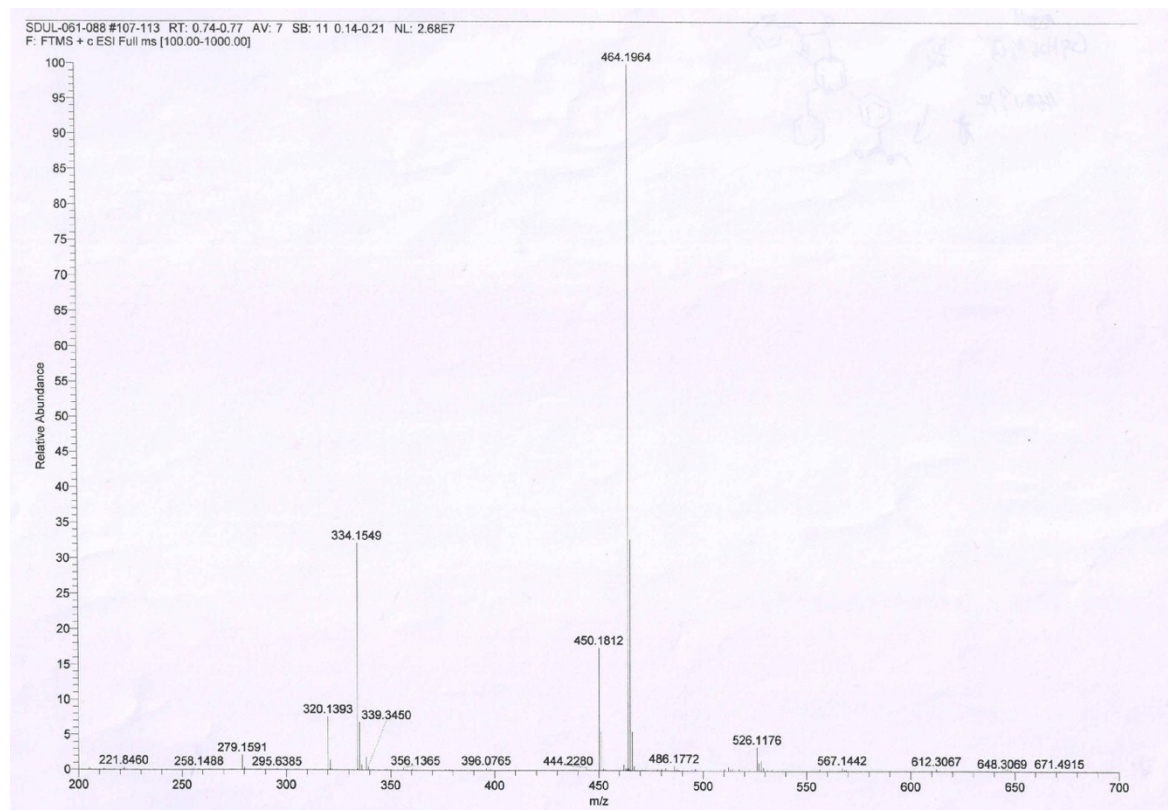
B5



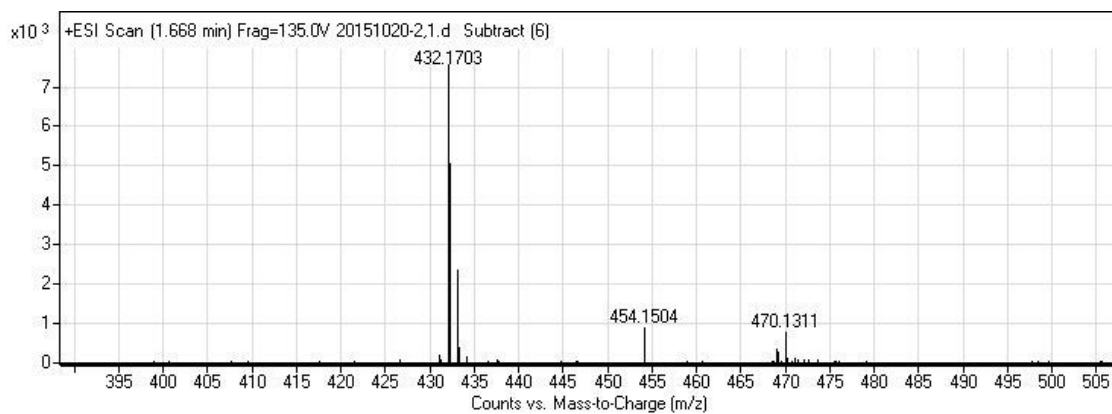
B6



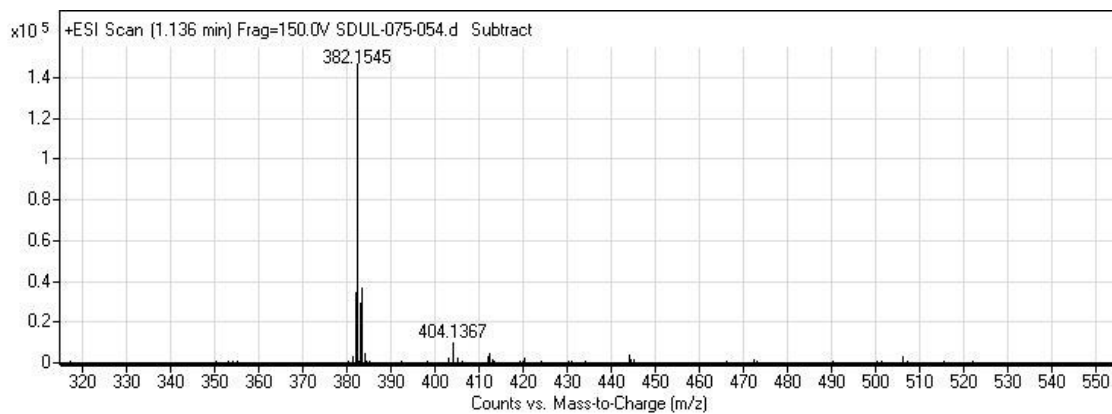
B7



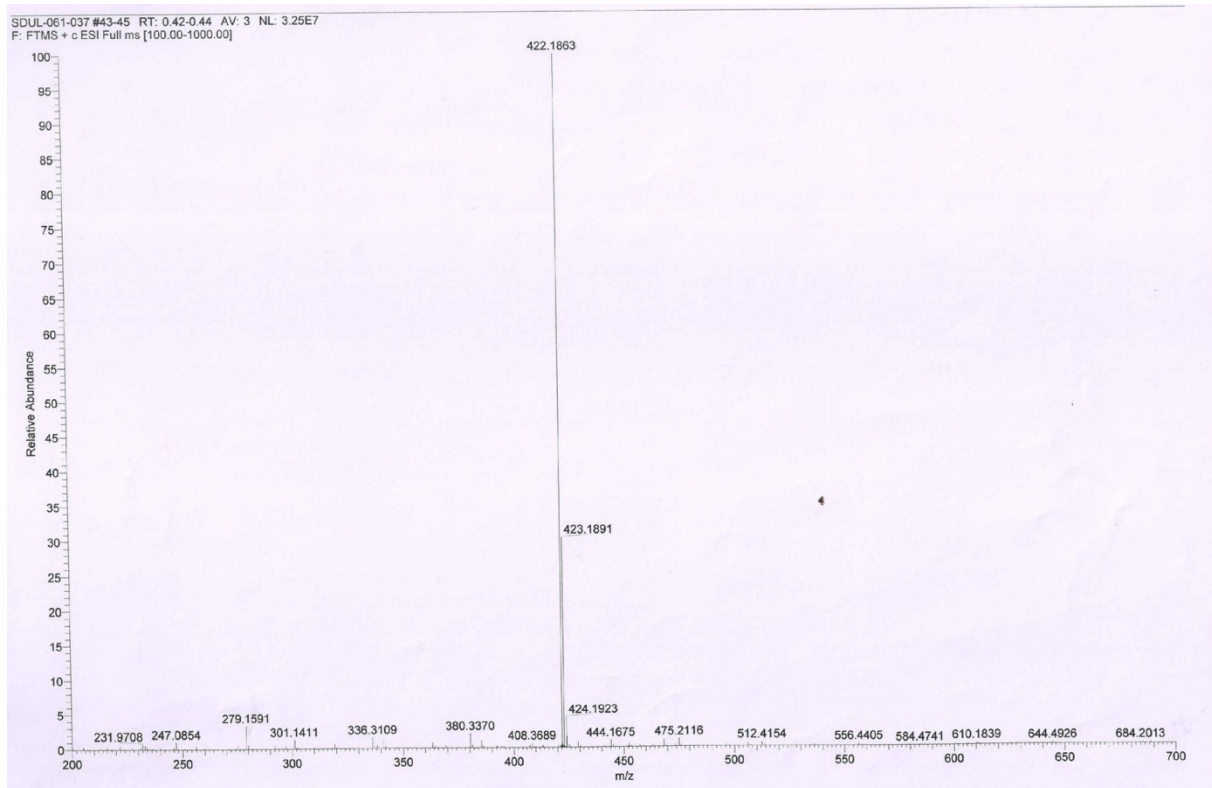
B8



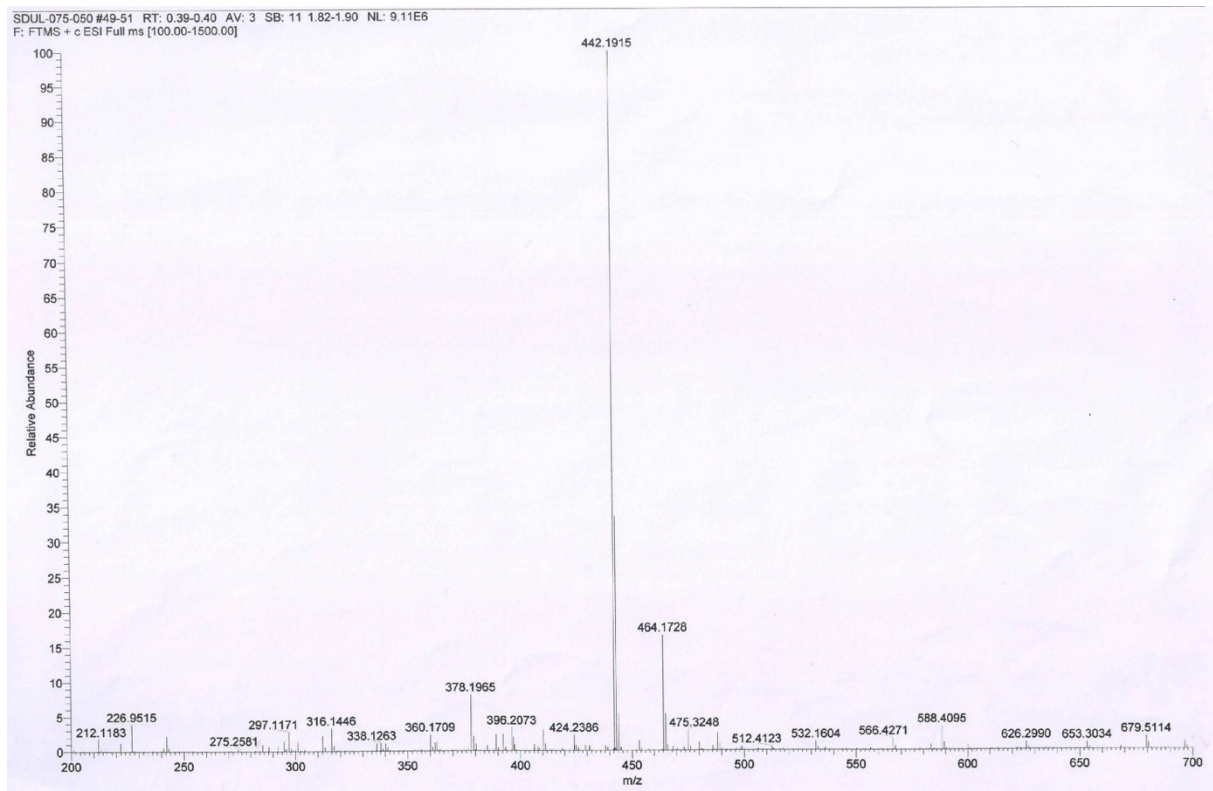
B9



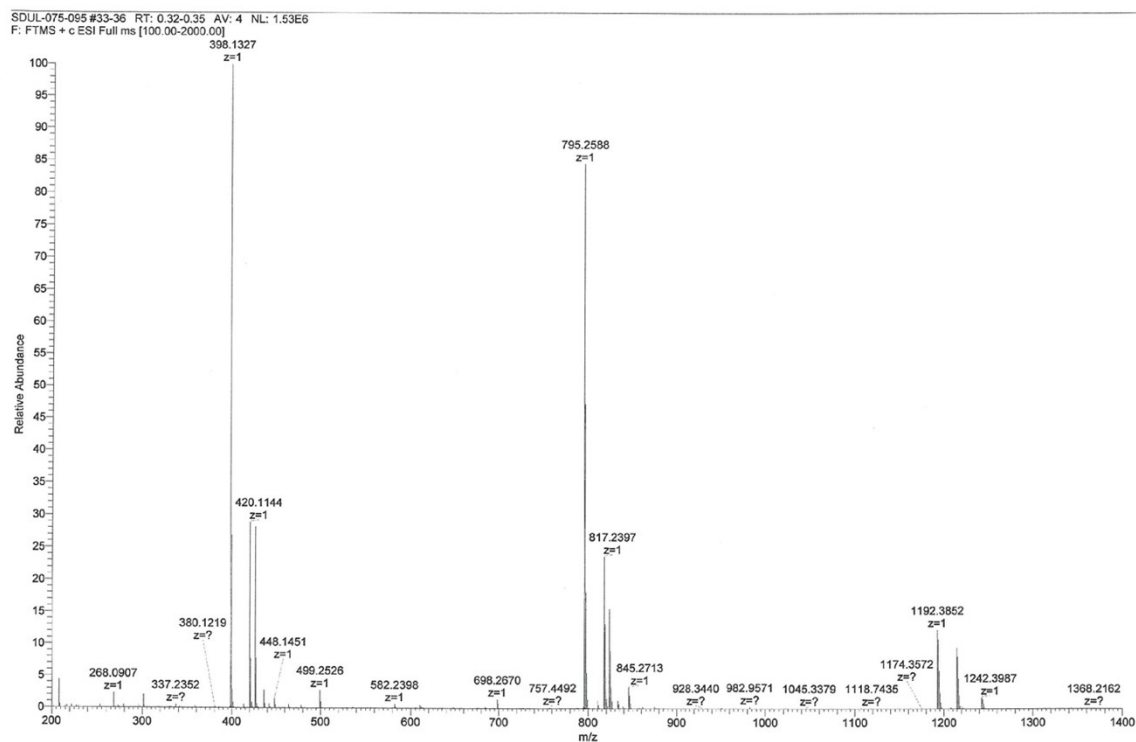
B10



B11



B12



B13

SDUL-061-019-#17-22 RT: 0.22-0.25 AV: 6 SB: 17 1.81-1.90 NL: 2.63E7
F: FTMS + c ESI Full ms [100.00-1000.00]

