

Supporting Information

Design, Synthesis and Evaluation of New Quinazolinone Derivatives that Inhibit Bloom's Syndrome Protein (BLM) Helicase, Trigger DNA damage at the Telomere Region and Synergize with PARP Inhibitors

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Table S1. Binding affinity of **1** to wild-type and mutant BLM using ITC assay in our previous report. “n.d.” in the table meant the K_D value was not obtained by fitting.

Comp.	1						
Variants	BLM ^{WT}	BLM ^{Y995A}	BLM ^{H996A}	BLM ^{M1111A}	BLM ^{E1143A}	BLM ^{I1168A}	DNA
K_D (μ M)	0.76	n.d.	1.40	n.d.	n.d.	6.54	n.d.

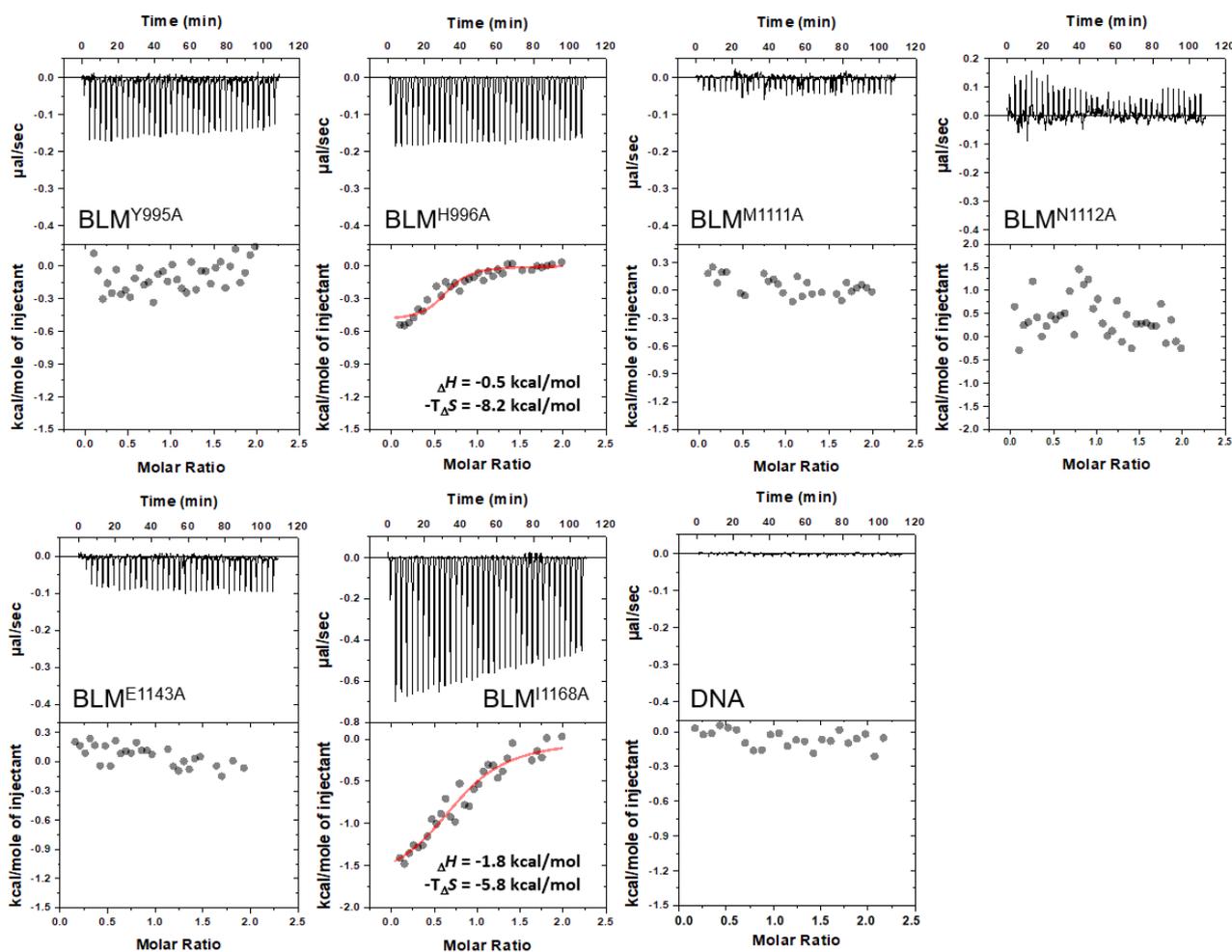


Figure S1. The ITC curves and fitted affinity of **9h** to mutated BLM proteins. The top panel showed the heat change upon ligand titration, the bottom panel showed the integrated data and ITC isotherm (red solid line) fitted by a single-site binding model.

Table S2. The cytotoxicity of compounds on HCT116 by MTT assay.

Compd.	IC₅₀ (μM)						
1	5.5	12f	4.2	1g	23.1	7h	n.d.
1f	25.0	13f	50.0	2g	12.5	8h	12.4
2f	10.3	14f	n.d. ^a	3g	20.5	9h	8.0
3f	5.9	15f	25.6	4g	n.d.	1i	5.7
4f	14.7	16f	13.5	5g	28.1	1j	2.9
5f	7.2	17f	25.0	6g	10.9	2j	3.1
6f	3.4	18f	n.d.	1h	5.3	3j	5.3
7f	6.8	19f	n.d.	2h	8.1	4j	1.6
8f	7.1	20f	2.2	3h	7.7	5j	5.2
9f	35.5	21f	23.7	4h	8.4		
10f	10.5	22f	12.4	5h	9.6		
11f	20.2	23f	n.d.	6h	15.6		

^an.d.: IC₅₀ value could not be fitted within 50 μM.

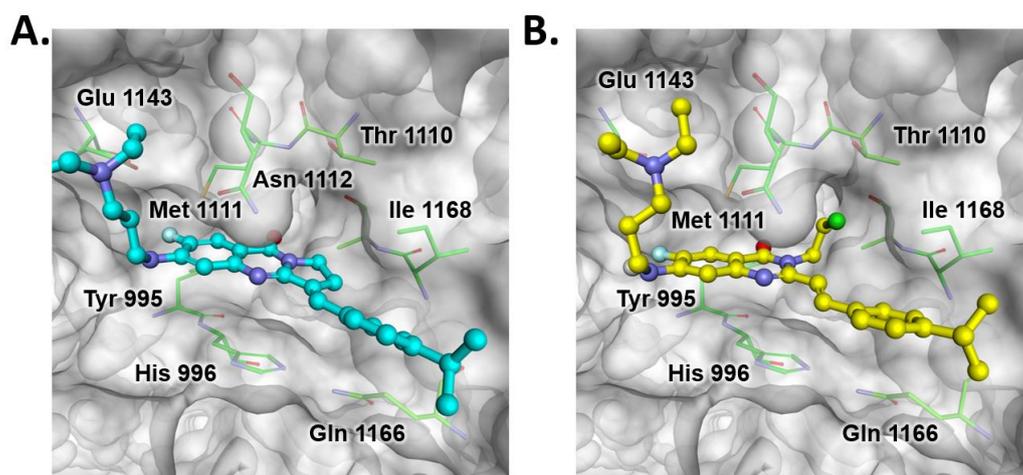


Figure S2. Predicted binding mode of **1** (A) and **9h** (B) with BLM (PDB code 4cgz), the diagram showed the interaction between ligand **1** (cyan) or **9h** (yellow) and predicted residues (green), the surface of protein was shown in grey.

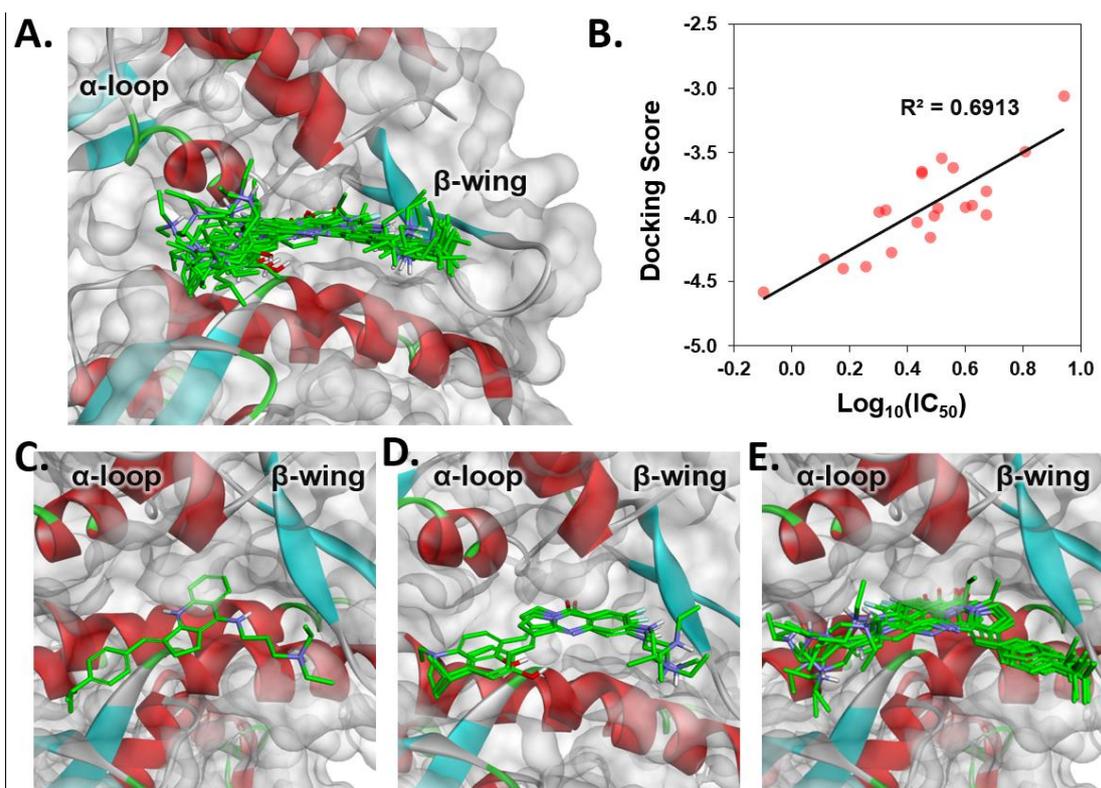


Figure S3. (A) Predicted binding mode of all the BLM inhibitors; (B) Plot of $\text{Log}_{10}(\text{IC}_{50})$ and docking score; (C) Predicted binding mode of **1j**; (D) Predicted binding mode of **12f**, **20f** and **5h**; (E) Predicted binding mode of **3f**, **4f**, **6f**, **22f**, **4h** and **6h**.

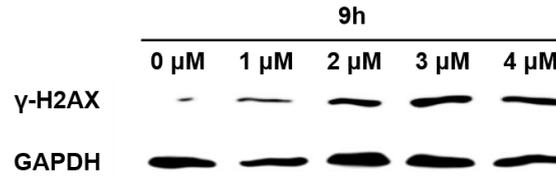


Figure S4. DNA Damage Induced by BLM inhibitors. The expression level of γ -H2AX with the treatment of **9h** (0-4 μ M) in HCT116 cells for 24 h, GAPDH was used as a control.

Table S3. The combination index (CI) of **9h** or **1** with PARP inhibitors, chemotherapy agent, and telomerase inhibitor measured by CompuSyn software.

		Niraparib		Olaparib		Cisplatin		BIBR1532	
		12.5 μ M	25.0 μ M						
9h	1.0 μ M	0.42	0.64	0.47	0.72	0.58	0.79	0.57	0.84
	2.0 μ M	0.57	0.80	0.65	0.68	0.64	0.77	0.49	0.72
1	1.0 μ M	0.84	0.98	0.67	0.65	-	-	-	-
	2.0 μ M	1.23	1.21	0.97	1.03	-	-	-	-

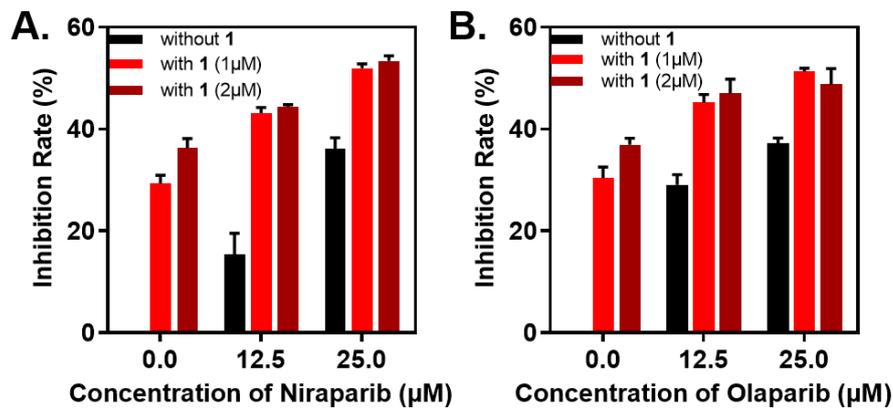


Figure S5. The cytotoxicity of **9h** in combined treatment with PARP inhibitor **Niraparib** (A) and **Olaparib** (B).

The data are presented as the mean + SEM (n = 3, biological replicates).

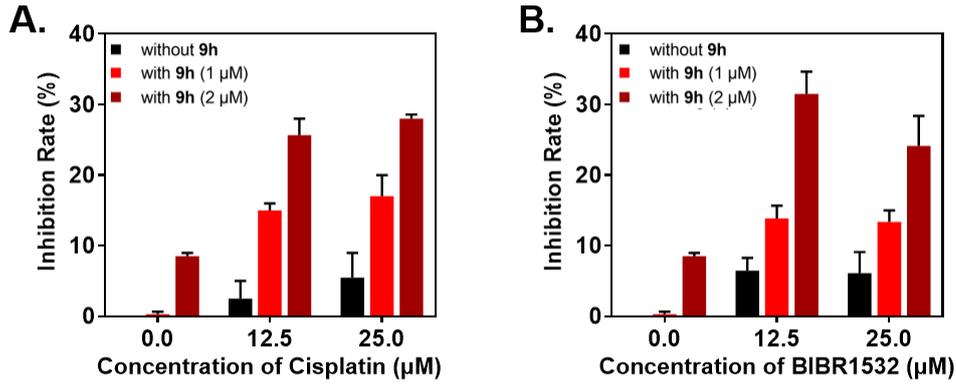


Figure S6. Compound **9h** enhanced sensitivity of chemotherapy agent and telomerase inhibitor to HCT116 cells for 48h. The cytotoxicity of **9h** in combined treatment with chemotherapy agent Cisplatin (A) and telomerase inhibitor **BIBR1532** (B). The data are presented as the mean + SEM (n = 3, biological replicates).

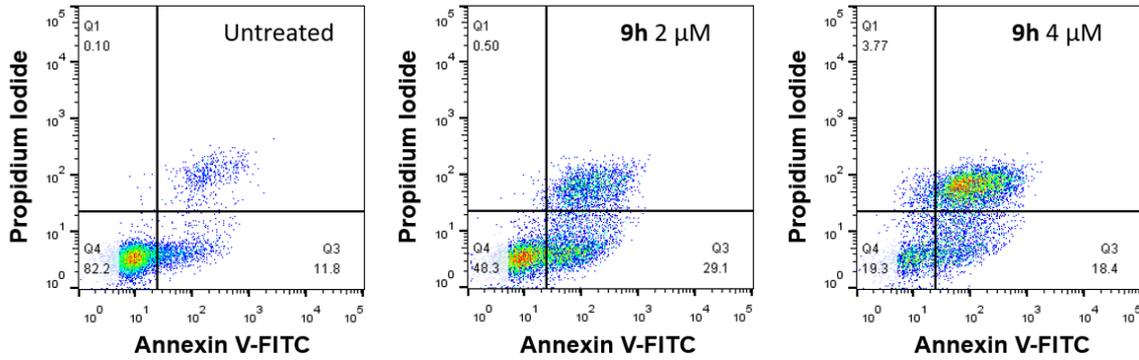


Figure S7. The apoptosis of HCT116 cells treated with **9h** in Annexin V-FITC/PI detection.

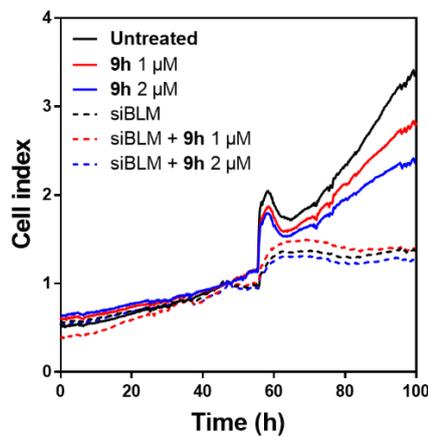


Figure S8. The proliferative ability in HCT116 and siBLM HCT116 cells treated with **9h** in RTCA assay.

Methods and Materials

Table S4. DNA oligomers used in this study

Oligomer name	Sequence (from 5' to 3')
<i>Biotin forked-DNA-A</i>	TTTTTTTTTTTCGTACCCGATGTGTTTCGTTTC
<i>forked-DNA-S</i>	GAACGAACACATCGGGTACGTTTTTTTTTTT
<i>BLM siRNA</i>	AAGAGAGTGGAGTGGCTTTTG

Table S5. Primers used in this study

Primer name	Sequence (from 5' to 3')
<i>Cleavage efficiency-A</i>	ATGGTGAGCAAGGGCGAGGA
<i>Cleavage efficiency-S</i>	CTGCACGCCGTAGGTCAGGGT
<i>DSB site on model (-92bp)-A</i>	GGCGTAAATTGTAAGCGTTAATCTG
<i>DSB site on model (-92bp)-S</i>	CCAAATGTGTCAGTTTCATAGCCT
<i>DSB site on model (+559bp)-A</i>	AGAACCACAAAGTGGGAATCAAG
<i>DSB site on model (+559bp)-S</i>	CCAAATTAAGGGCCAGCTCAT
<i>DSB site on model (+930bp)-A</i>	CCAGAAAGCGAAGGAGCAAAG
<i>DSB site on model (+930bp)-S</i>	ACGTCTCACTAGTCTCGTGCAGAT
<i>DSB site on model (+2351bp)-A</i>	GCTAACCATGTTTCATGCCTTCTT
<i>DSB site on model (+2351bp)-S</i>	CGGCGGATCTGAATTCTTTG

Real-Time Cellular Analysis (RTCA)

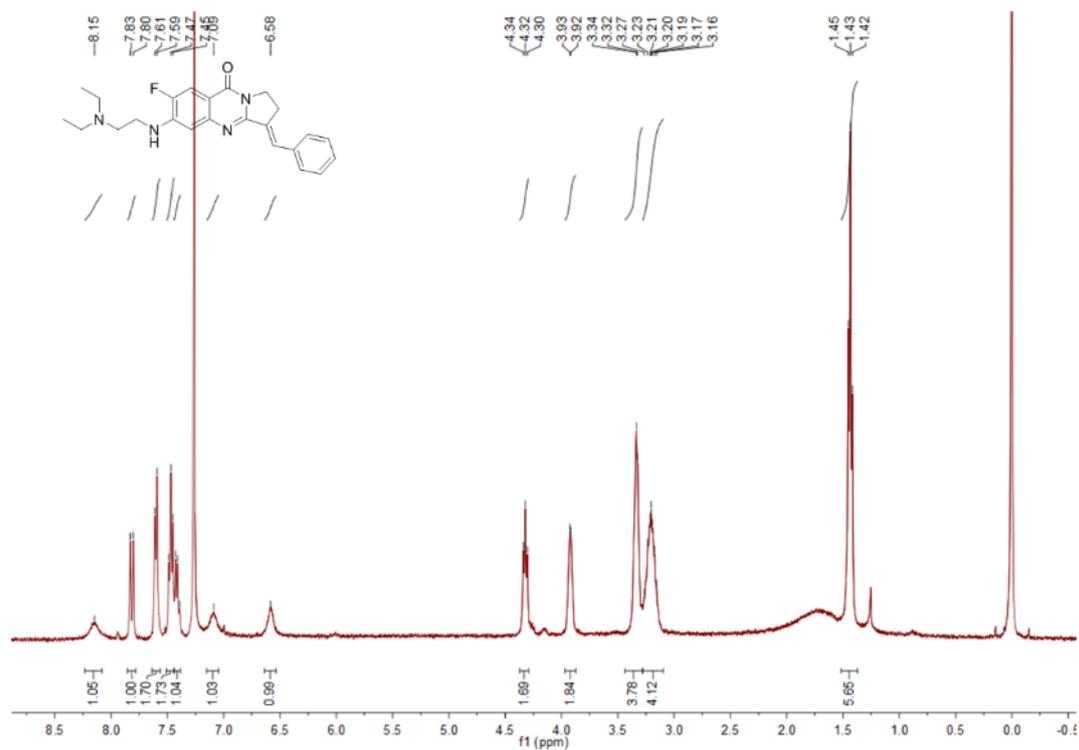
E-Plates were used in this study to monitor the growth of cells using the xCELLigence DP system (Roche Applied Science, Indianapolis, IN), because the gold microelectrode sensor could generate an electric field when a low voltage (< 20 mV) is applied between electrodes. HCT116 and BJ cells (2×10^3) were planted on each well in the E-Plate 16-well plates (Roche Applied Science, Indianapolis, IN) and the number of cells were detected every 15 min intervals. Cells were transfected with 50 nM BLM small interfering RNA at 12 h after plating and continue cultured for another 36 h. Results were calculated via the RTCA software 1.2 (Roche Applied Science, Indianapolis, IN) and exported to Microsoft Excel software (Microsoft, Redmond, WA) for analysis and normalization.

Polarimetric Analysis

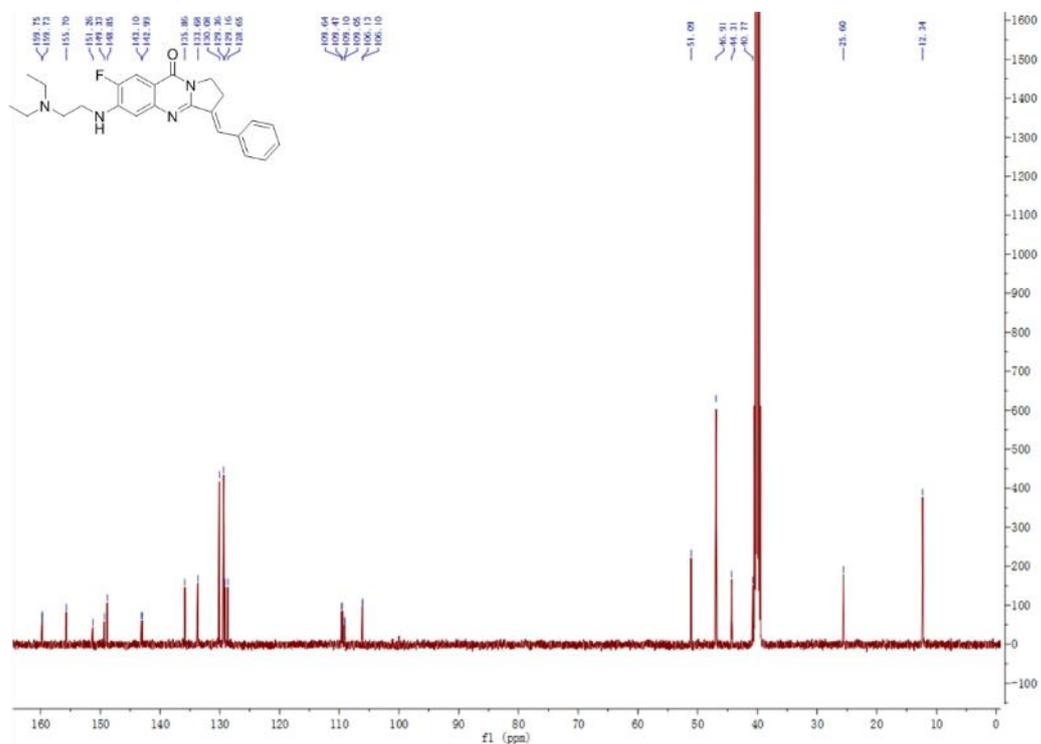
Polarimetric analysis was performed using an Anton Paar MCP200 polarimeter. The test conditions are shown as below. Solvent: methanol; Temperature: 20°C; Detection wavelength: 589 nm; Cell: 100 mm. Calculate the specific optical rotation with following equation: $[\alpha] = \alpha / (C \times L) \times 10,000$. In which: α is the optical rotation in degrees; C is the concentration in g/100 mL; L is the cell length in millimeters.

Characterization of Final Compounds

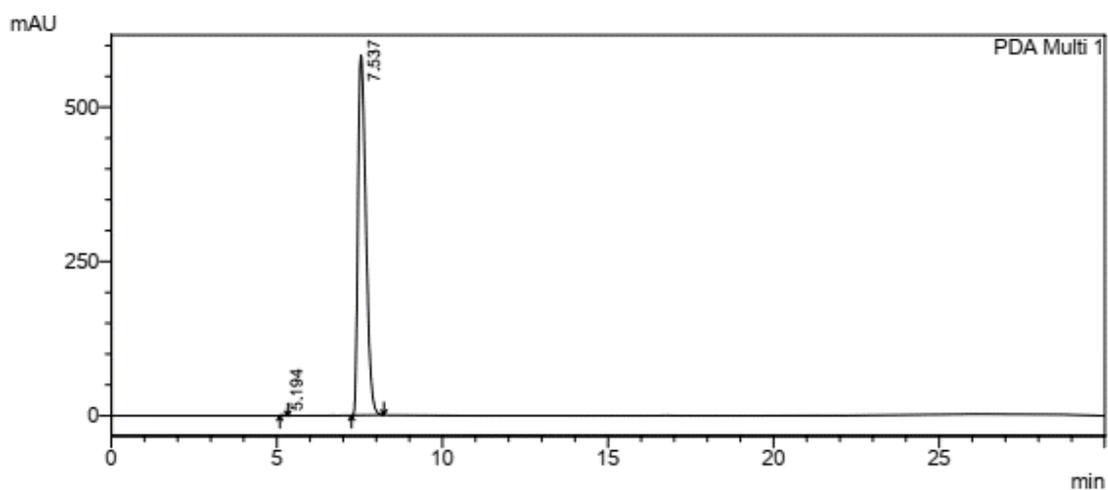
^1H NMR, ^{13}C NMR, HRMS and HPLC Spectrum of Final Compounds



^1H NMR spectrum of **1f**



^{13}C NMR spectrum of **1f**



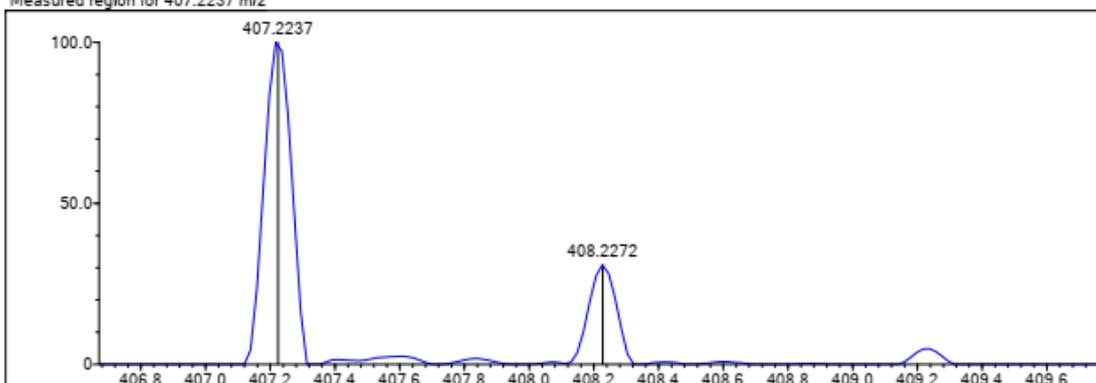
1 PDA Multi 1/254nm 4nm

PeakTable

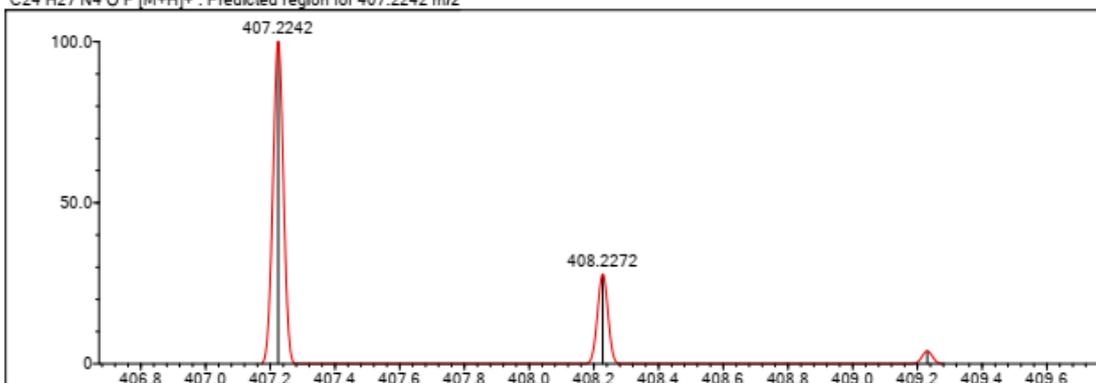
Peak#	Ret. Time	Area	Height	Area %	Height %
1	5.194	11213	1150	0.116	0.197
2	7.537	9692558	583401	99.884	99.803
Total		9703771	584552	100.000	100.000

HPLC analysis of **1f**

Measured region for 407.2237 m/z

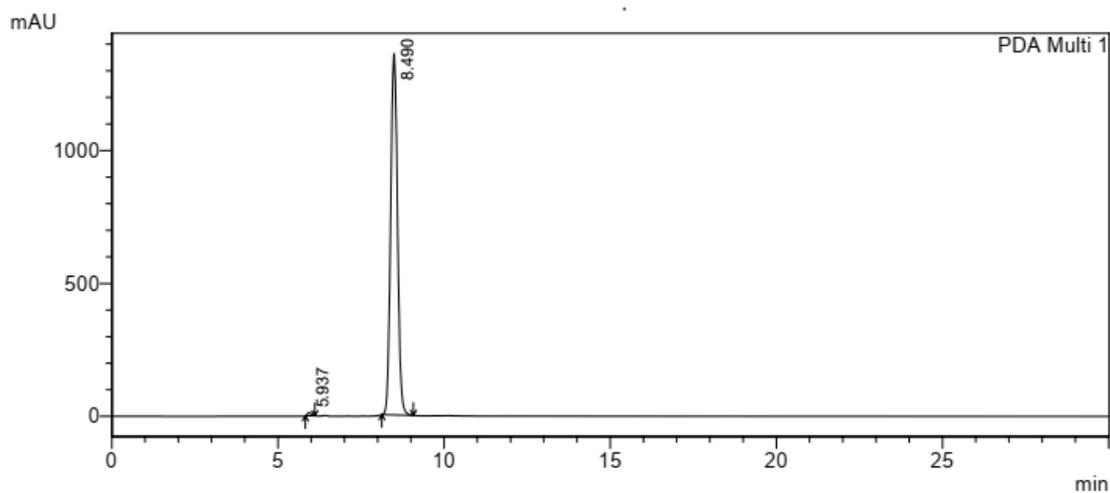


C24 H27 N4 O F [M+H]⁺ : Predicted region for 407.2242 m/z



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	84.58	C24 H27 N4 O F	[M+H] ⁺	407.2237	407.2242	-0.5	-1.23	85.06	13.0

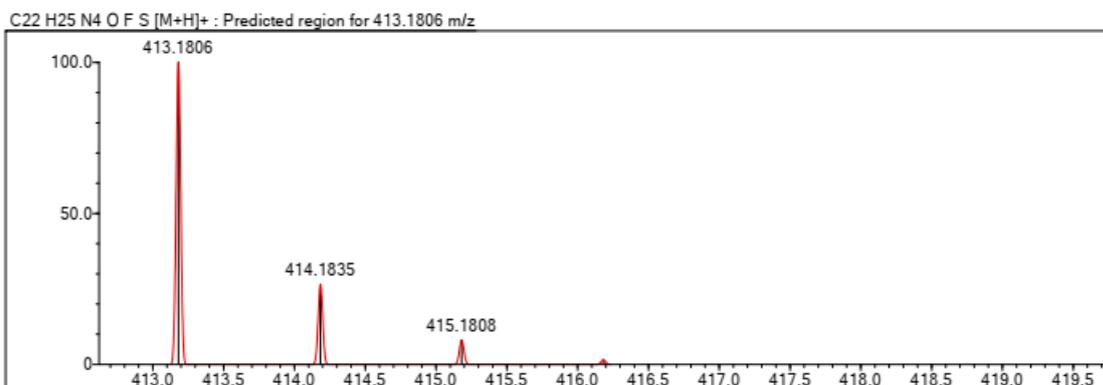
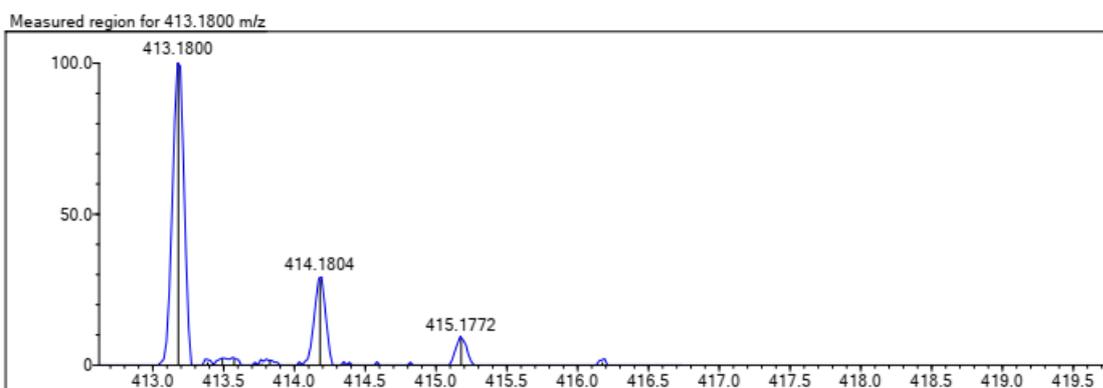
HRMS spectrum of **1f**



PeakTable

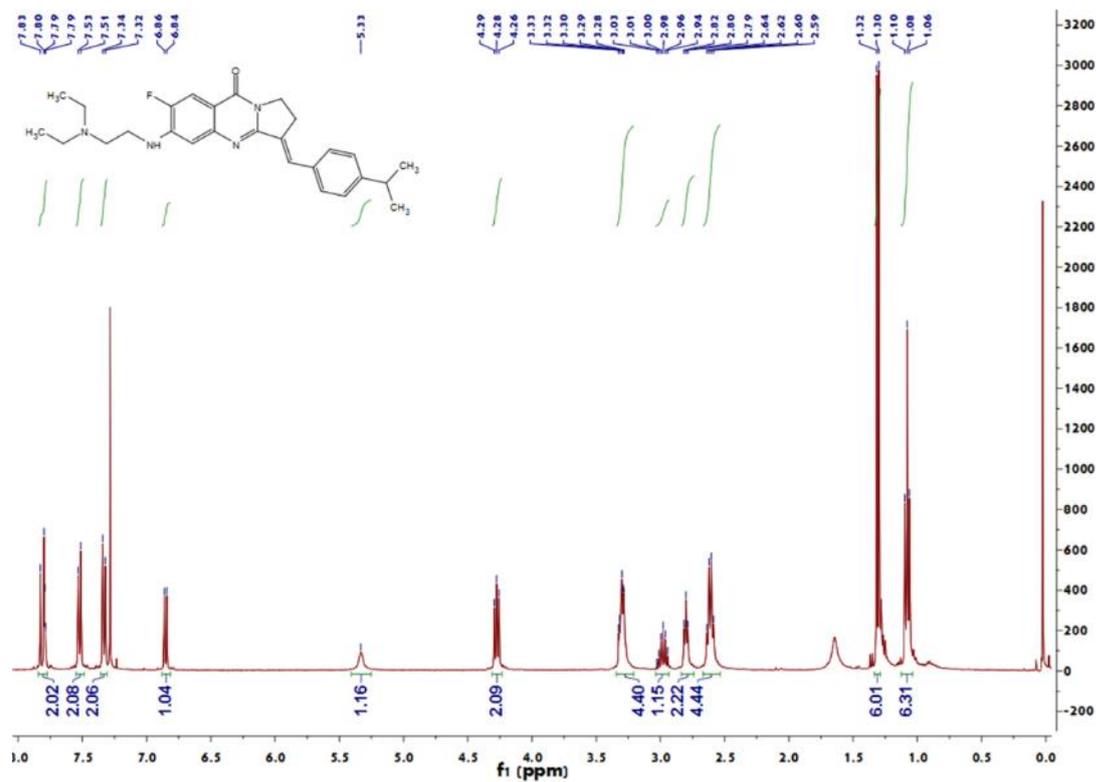
Peak#	Ret. Time	Area	Height	Area %	Height %
1	5.937	104412	11923	0.535	0.870
2	8.490	19395788	1359183	99.465	99.130
Total		19500200	1371106	100.000	100.000

HPLC analysis of 2f

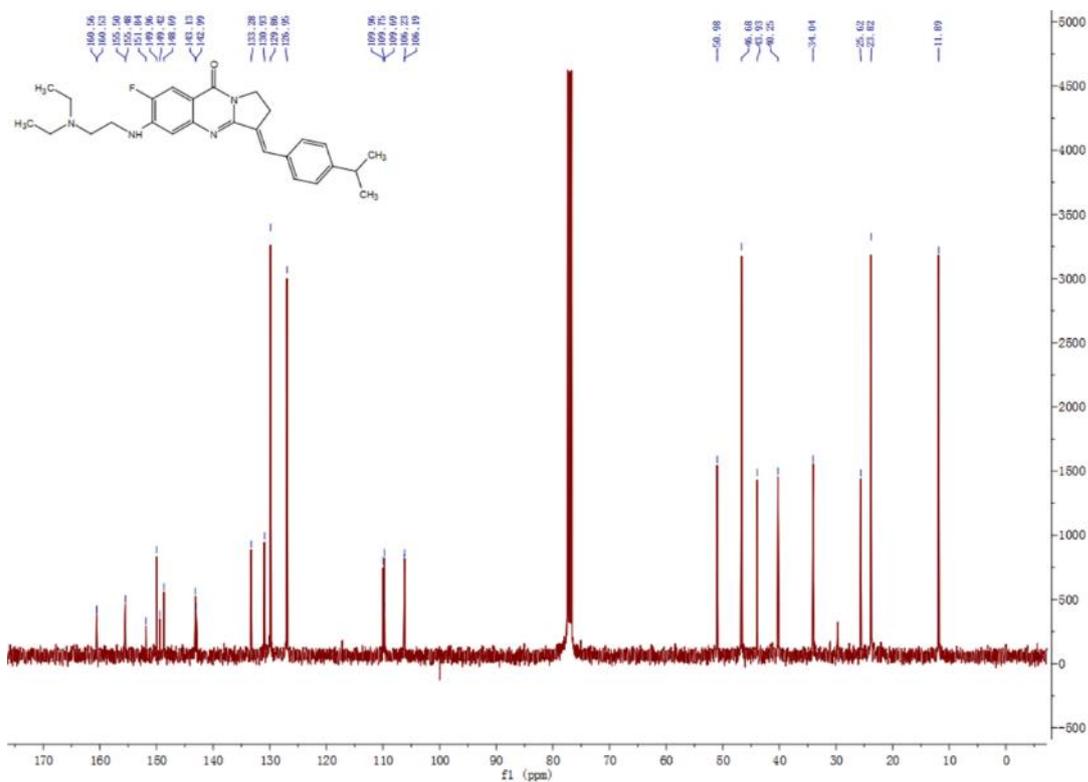


Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
2	80.36	C22 H25 N4 O F S	[M+H] ⁺	413.1800	413.1806	-0.6	-1.45	81.27	12.0

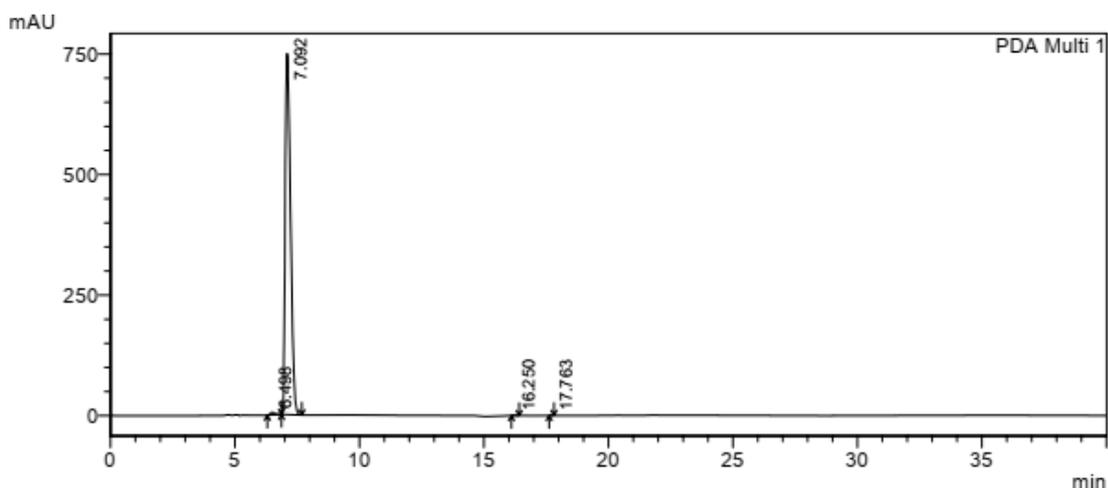
HRMS spectrum of 2f



^1H NMR spectrum of **3f**



^{13}C NMR spectrum of **3f**

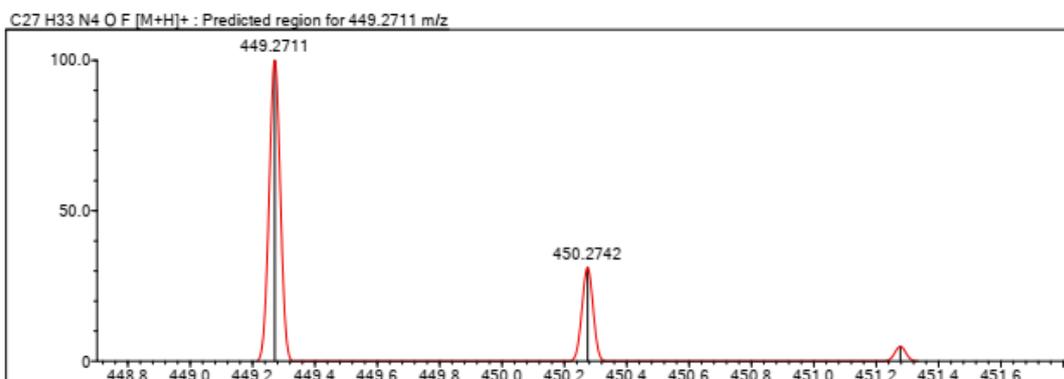
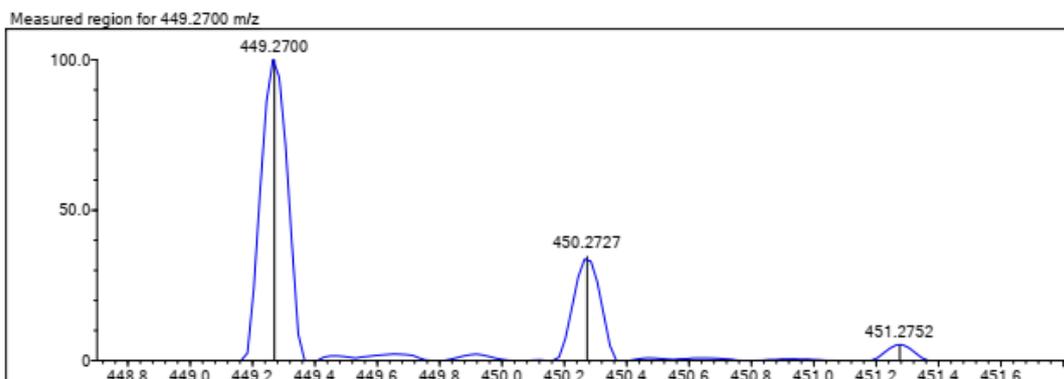


1 PDA Multi 1/254nm 4nm

PeakTable

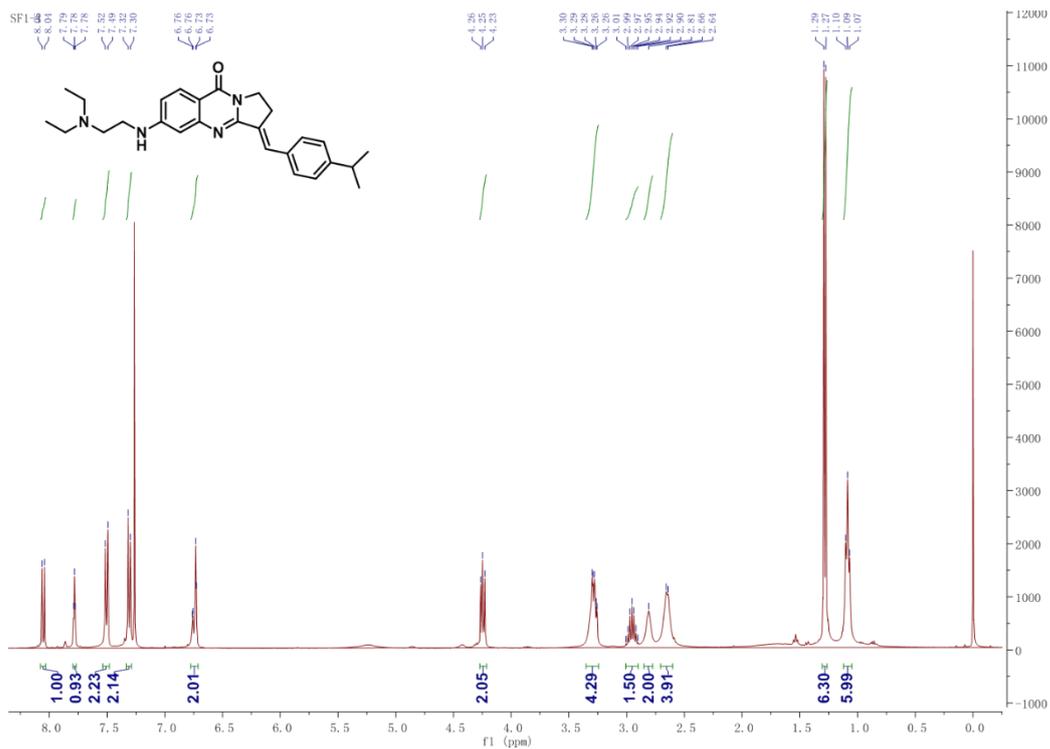
Peak#	Ret. Time	Area	Height	Area %	Height %
1	6.498	70702	5163	0.622	0.685
2	7.092	11283410	748459	99.338	99.259
3	16.250	4132	381	0.036	0.051
4	17.763	370	42	0.003	0.006
Total		11358615	754045	100.000	100.000

HPLC analysis of 3f

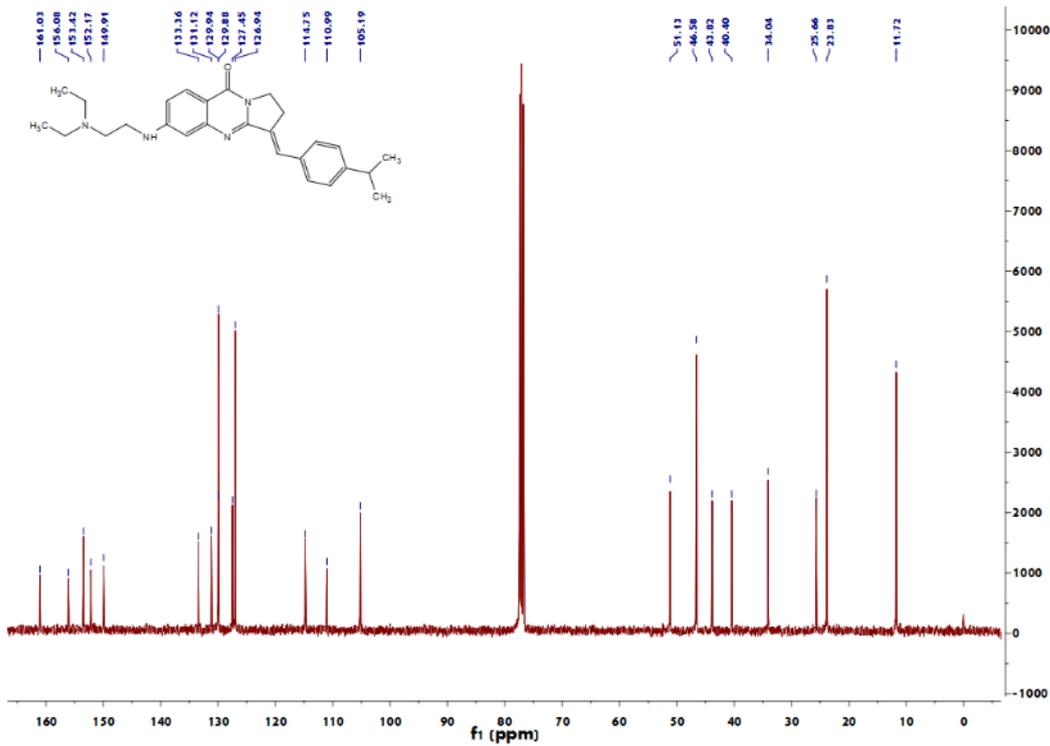


Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
3	84.72	C27 H33 N4 O F	[M+H] ⁺	449.2700	449.2711	-1.1	-2.45	87.91	13.0

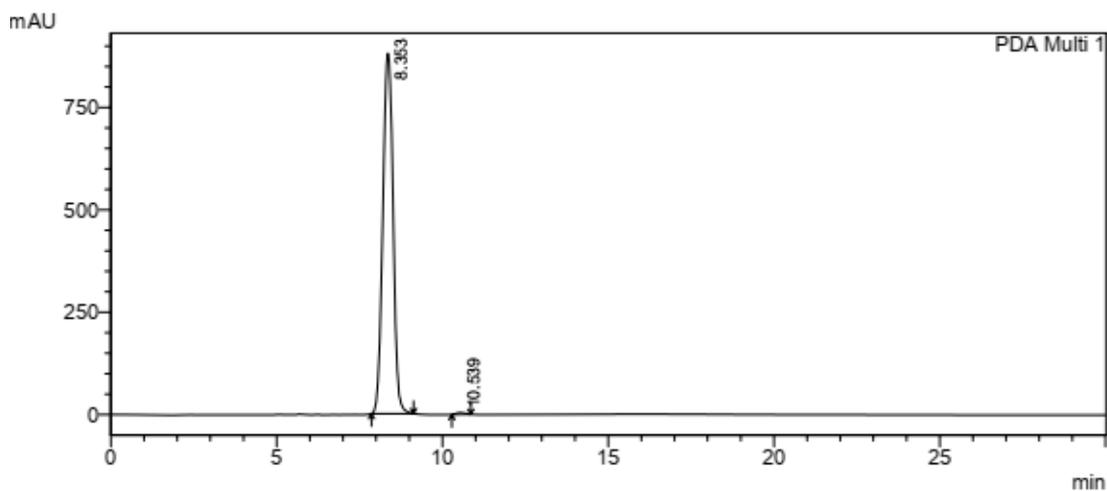
HRMS spectrum of 3f



¹H NMR spectrum of 4f



¹³C NMR spectrum of 4f



1 PDA Multi 1/254nm 4nm

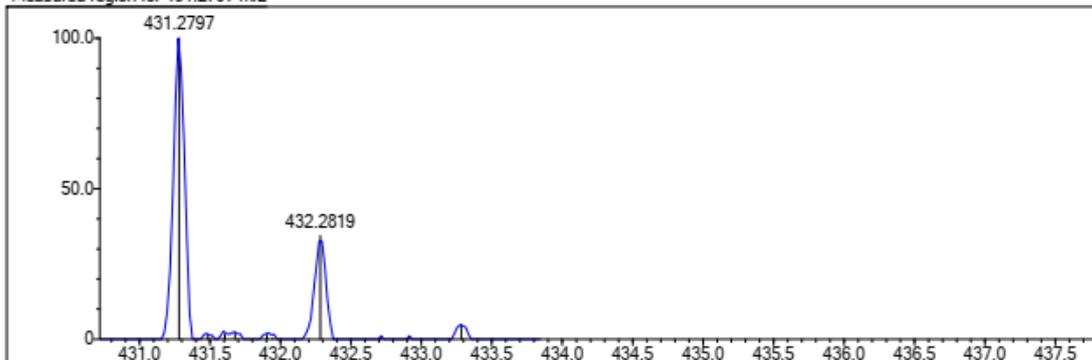
PeakTable

PDA Ch1 254nm 4nm

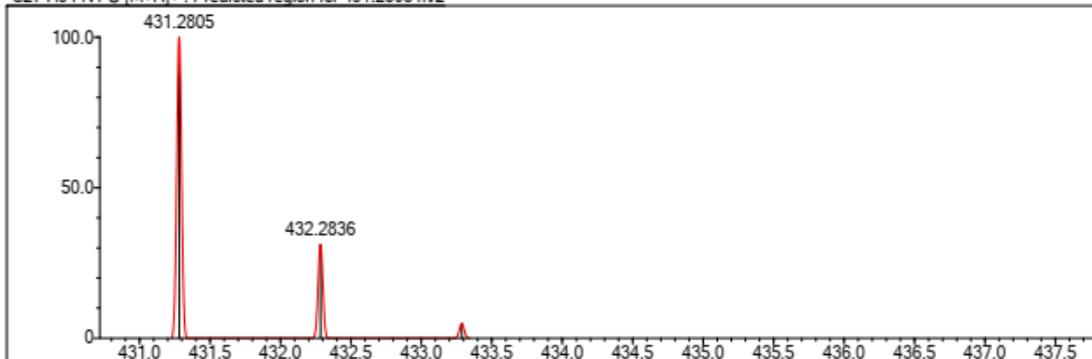
Peak#	Ret. Time	Area	Height	Area %	Height %
1	8.353	18930291	879821	99.564	99.495
2	10.539	82924	4461	0.436	0.505
Total		19013214	884283	100.000	100.000

HPLC analysis of 4f

Measured region for 431.2797 m/z

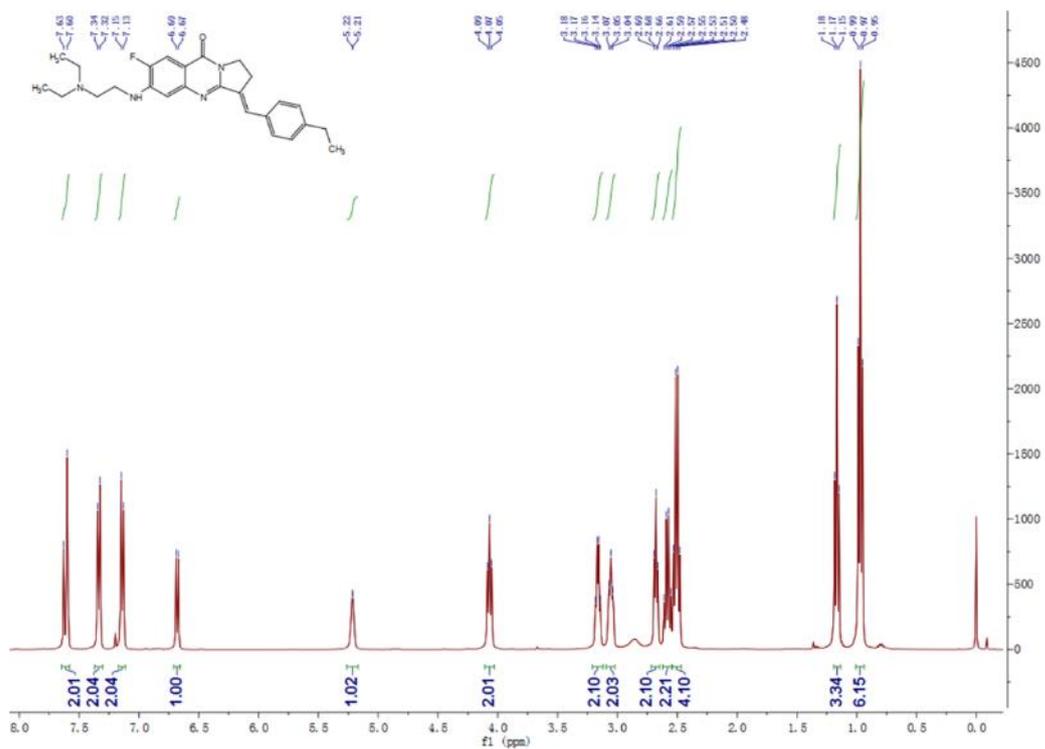


C27 H34 N4 O [M+H]⁺ : Predicted region for 431.2805 m/z

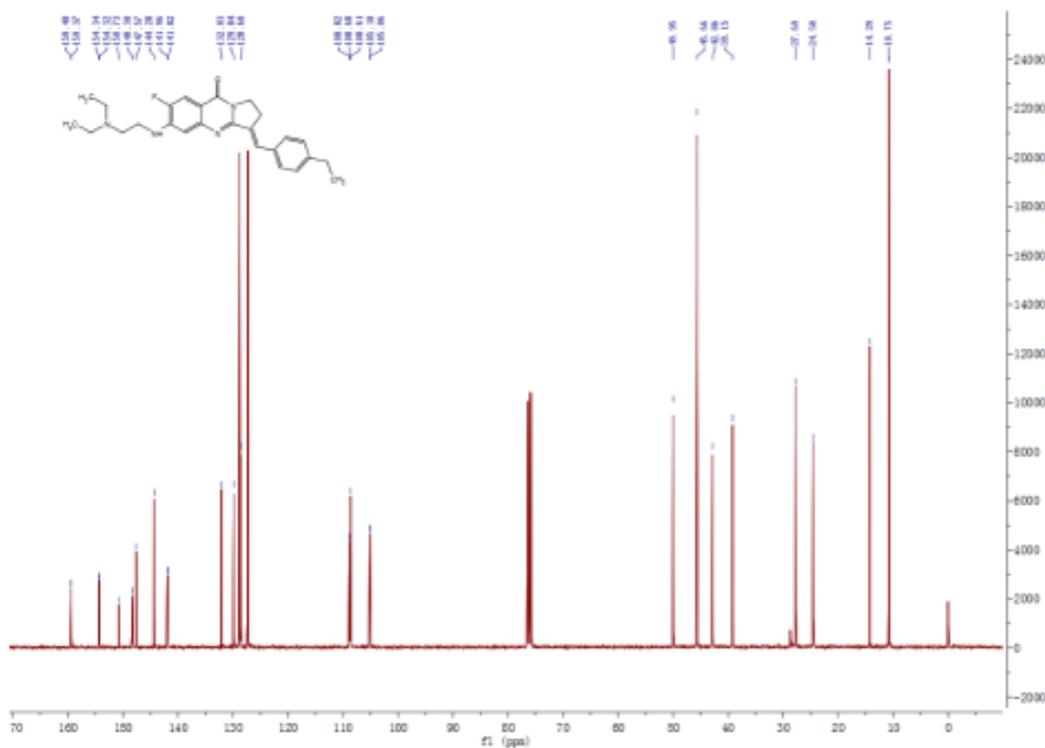


Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
2	88.27	C27 H34 N4 O	[M+H] ⁺	431.2797	431.2805	-0.8	-1.85	90.18	13.0

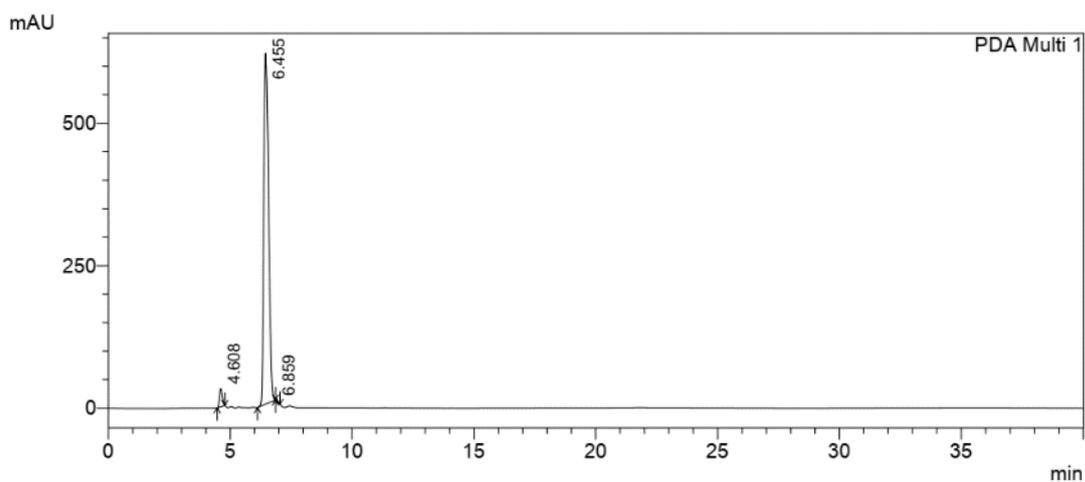
HRMS spectrum of 4f



¹H NMR spectrum of **5f**



¹³C NMR spectrum of **5f**



1 PDA Multi 1/254nm 4nm

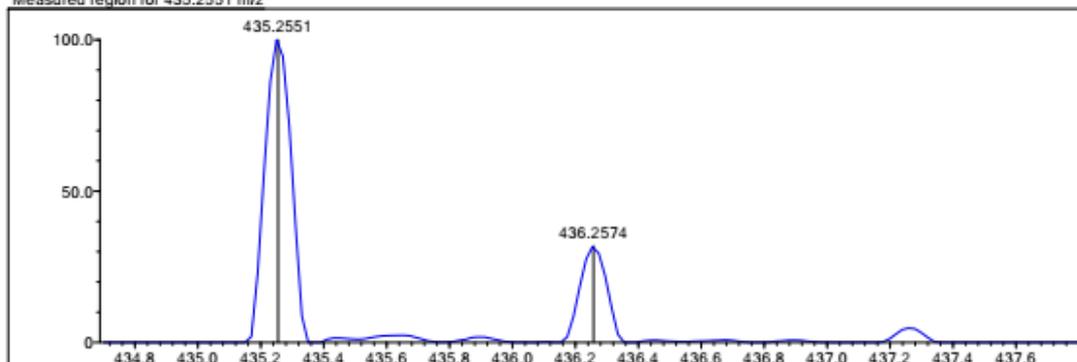
PeakTable

PDA Ch1 254nm 4nm

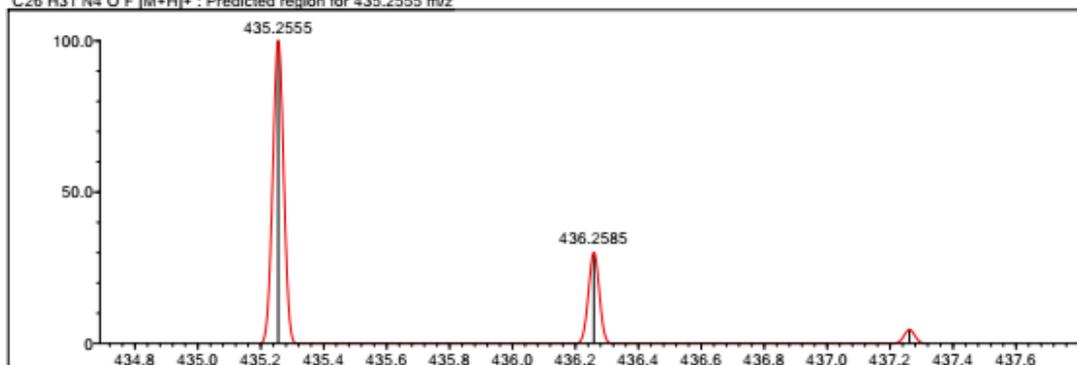
Peak#	Ret. Time	Area	Height	Area %	Height %
1	4.608	265636	31867	3.052	4.922
2	6.455	8431781	615620	96.863	95.078
3	6.859	7393	0	0.085	0.000
Total		8704810	647487	100.000	100.000

HPLC analysis of 5f

Measured region for 435.2551 m/z

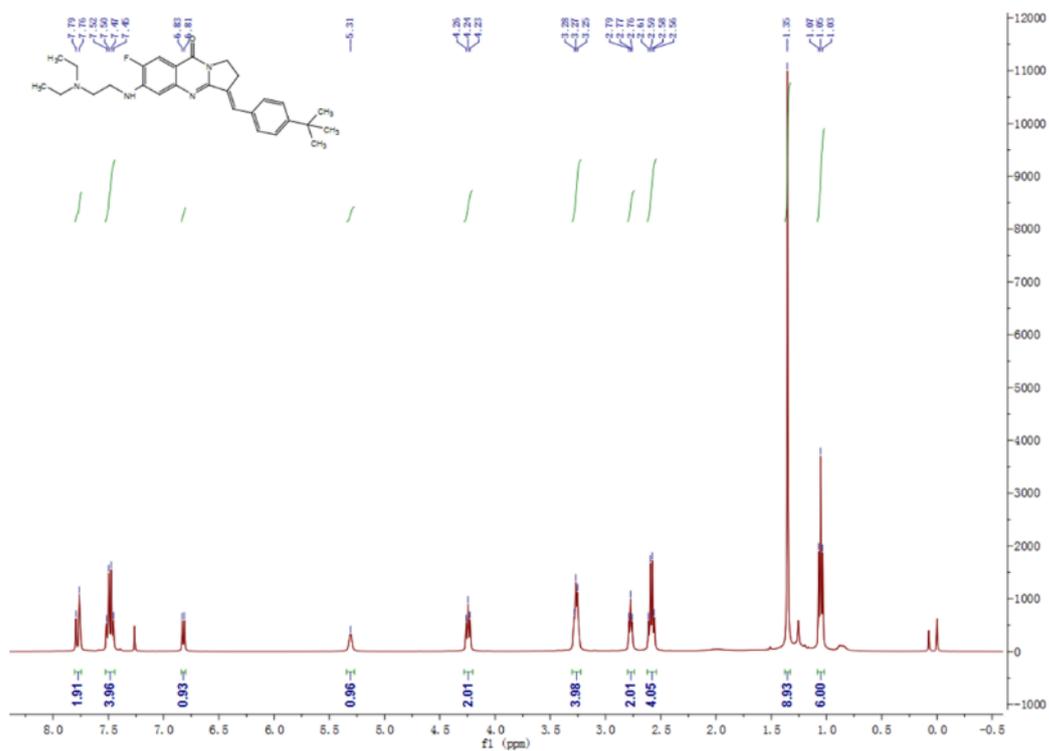


C26 H31 N4 O F [M+H]⁺ : Predicted region for 435.2555 m/z

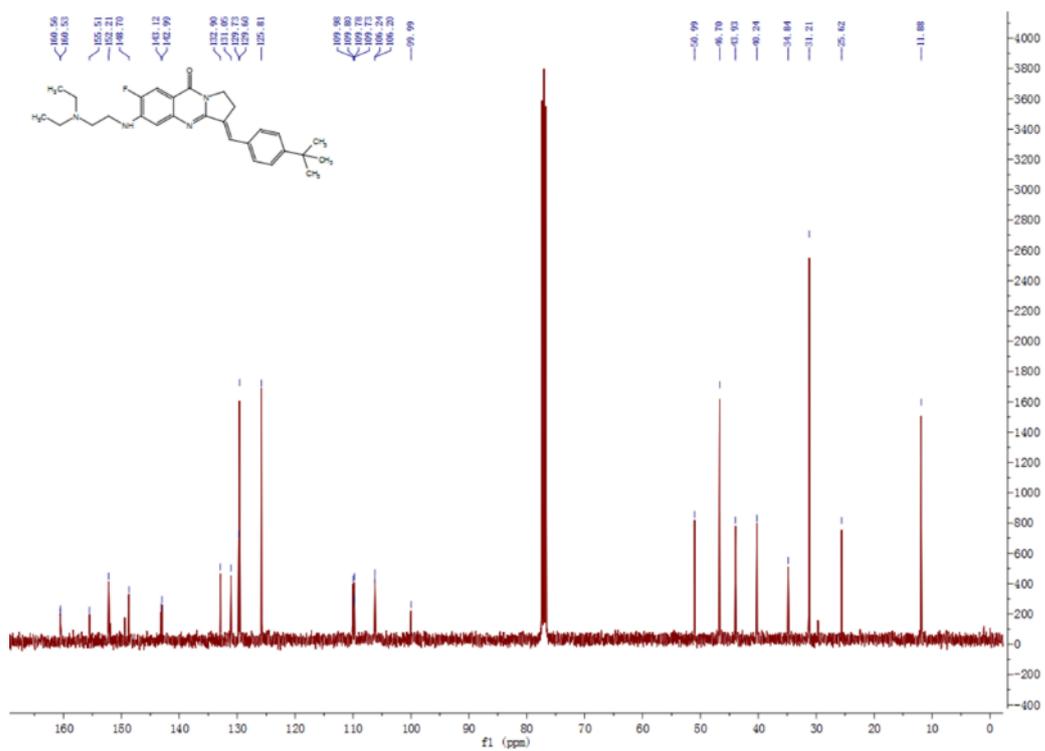


Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	80.24	C26 H31 N4 O F	[M+H] ⁺	435.2551	435.2555	-0.4	-0.92	80.24	13.0

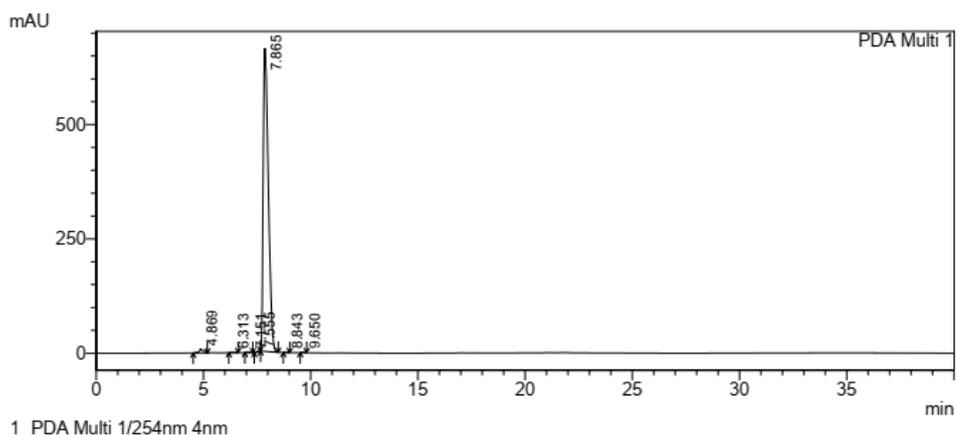
HRMS spectrum of 5f



¹H NMR spectrum of **6f**



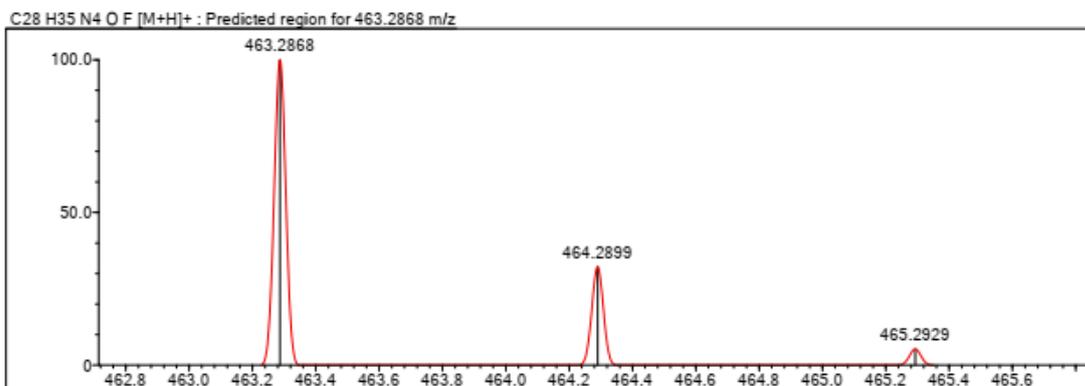
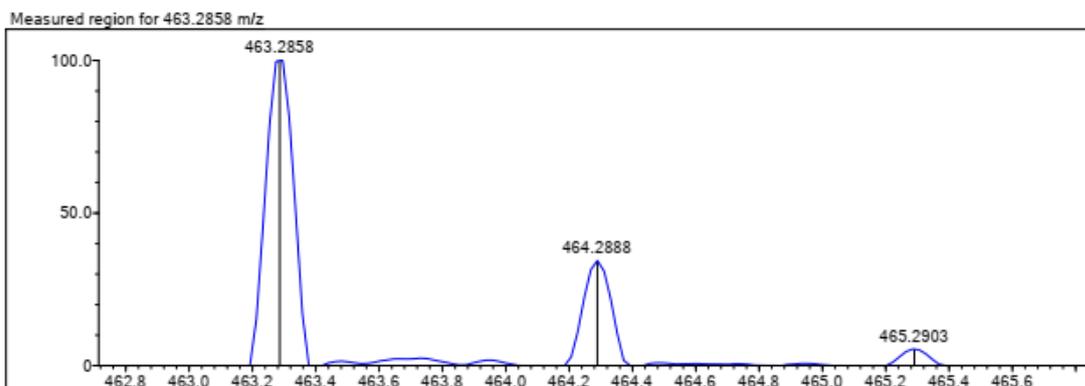
¹³C NMR spectrum of **6f**



PeakTable

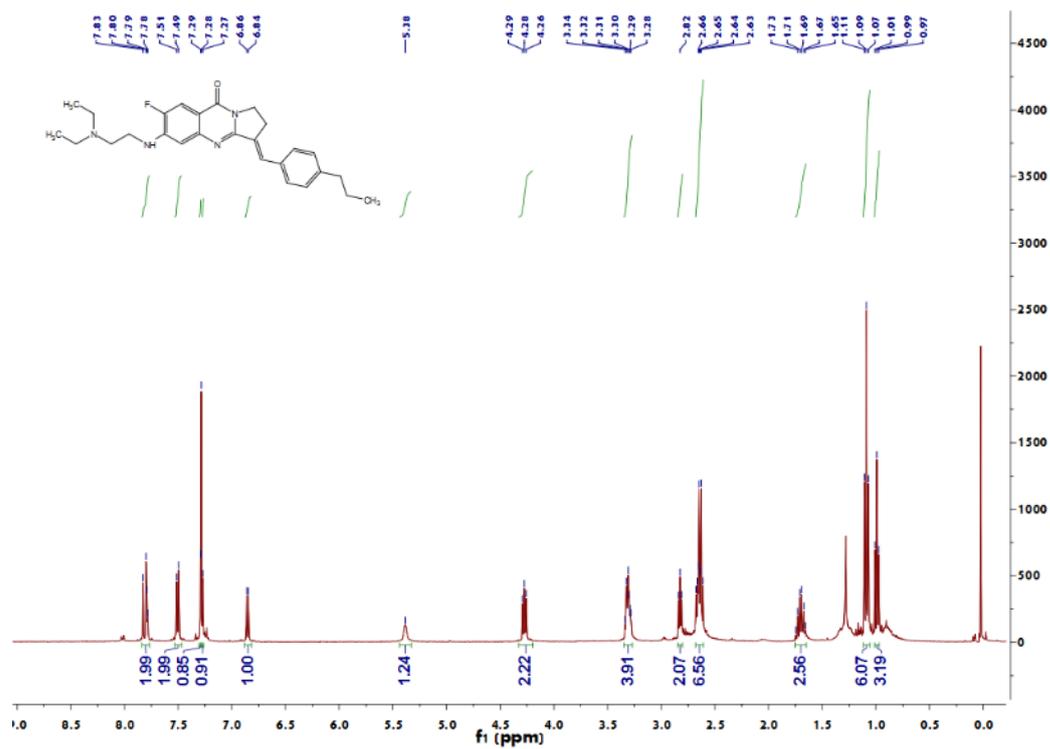
Peak#	Ret. Time	Area	Height	Area %	Height %
1	4.869	121561	8901	1.085	1.319
2	6.313	7467	408	0.067	0.060
3	7.151	7808	555	0.070	0.082
4	7.555	12842	1664	0.115	0.247
5	7.865	11039538	662632	98.575	98.157
6	8.843	3512	332	0.031	0.049
7	9.650	6388	580	0.057	0.086
Total		11199137	675074	100.000	100.000

HPLC analysis of 6f

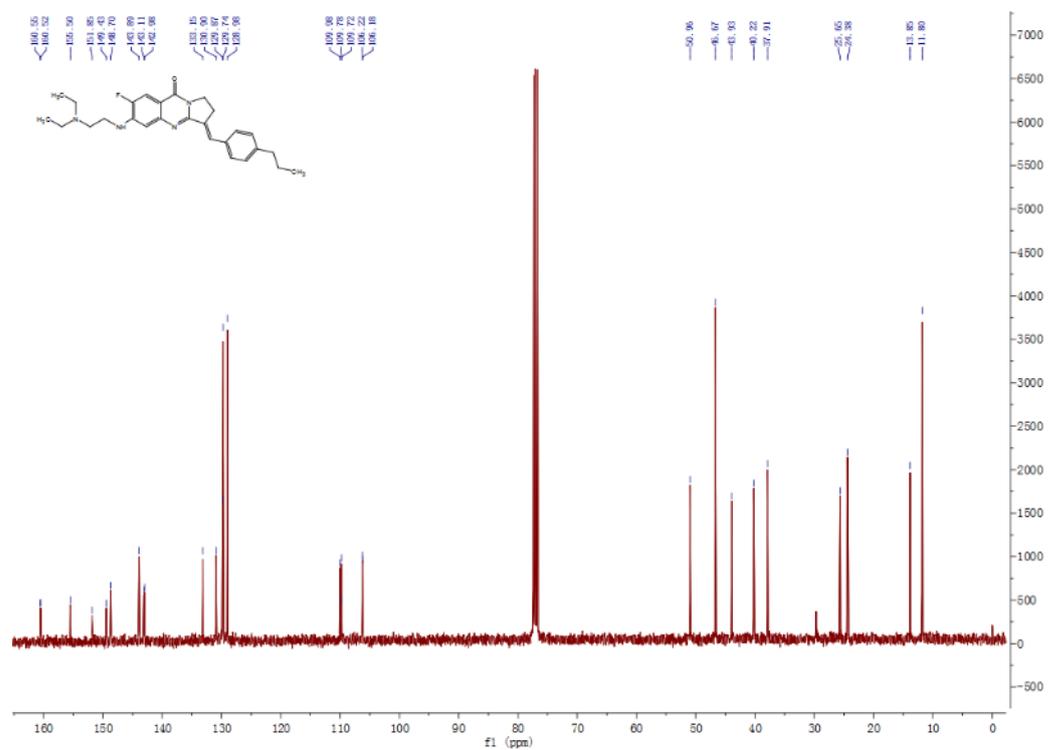


Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Isot	DBE
3	86.84	C28 H35 N4 O F	[M+H] ⁺	463.2858	463.2868	-1.0	-2.16	89.43	13.0

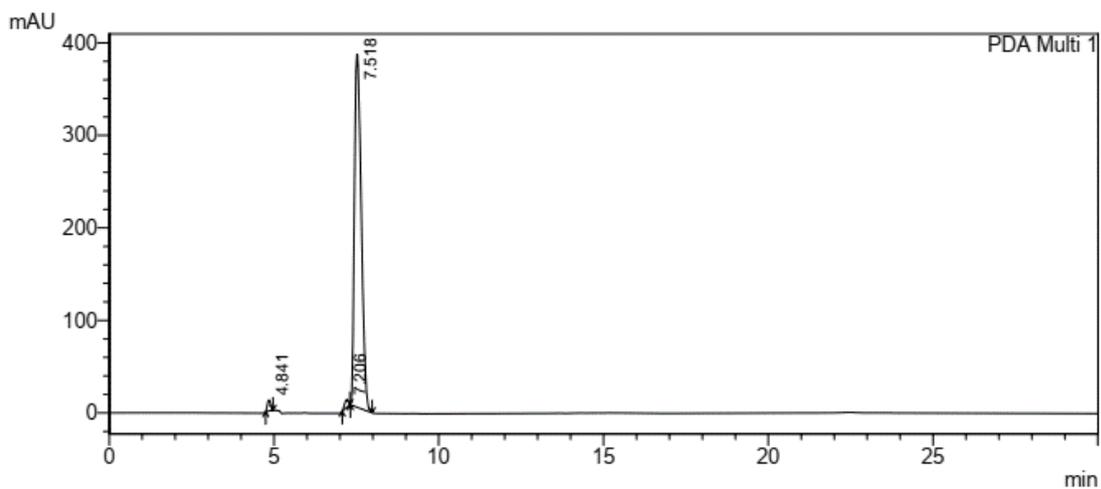
HRMS spectrum of 6f



¹H NMR spectrum of 7f



¹³C NMR spectrum of 7f



1 PDA Multi 1/254nm 4nm

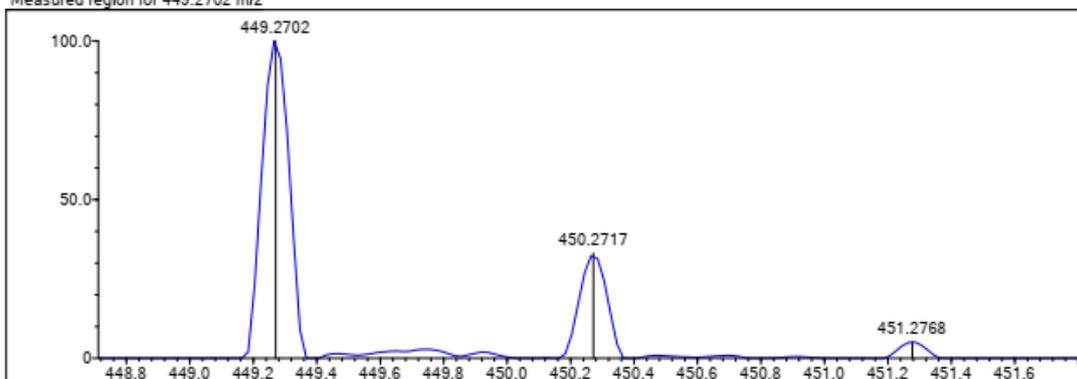
PeakTable

PDA Ch1 254nm 4nm

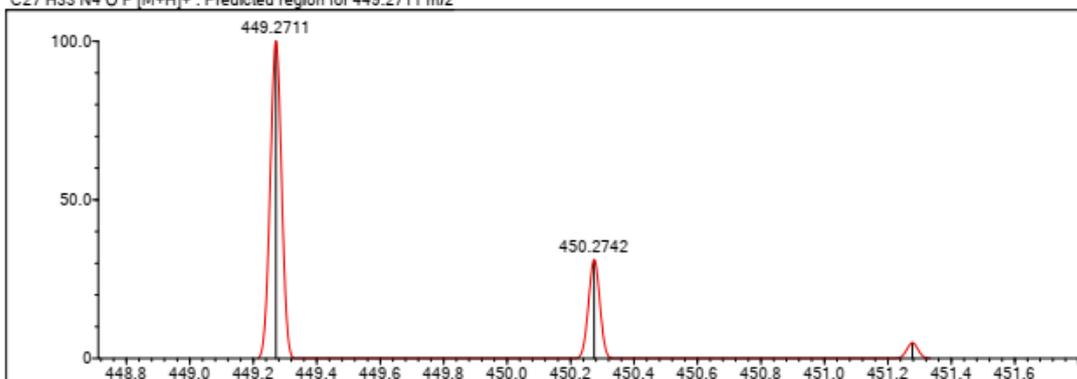
Peak#	Ret. Time	Area	Height	Area %	Height %
1	4.841	84844	11828	1.499	2.943
2	7.206	68892	8173	1.217	2.034
3	7.518	5507293	381891	97.284	95.023
Total		5661029	401892	100.000	100.000

HPLC analysis of 7f

Measured region for 449.2702 m/z

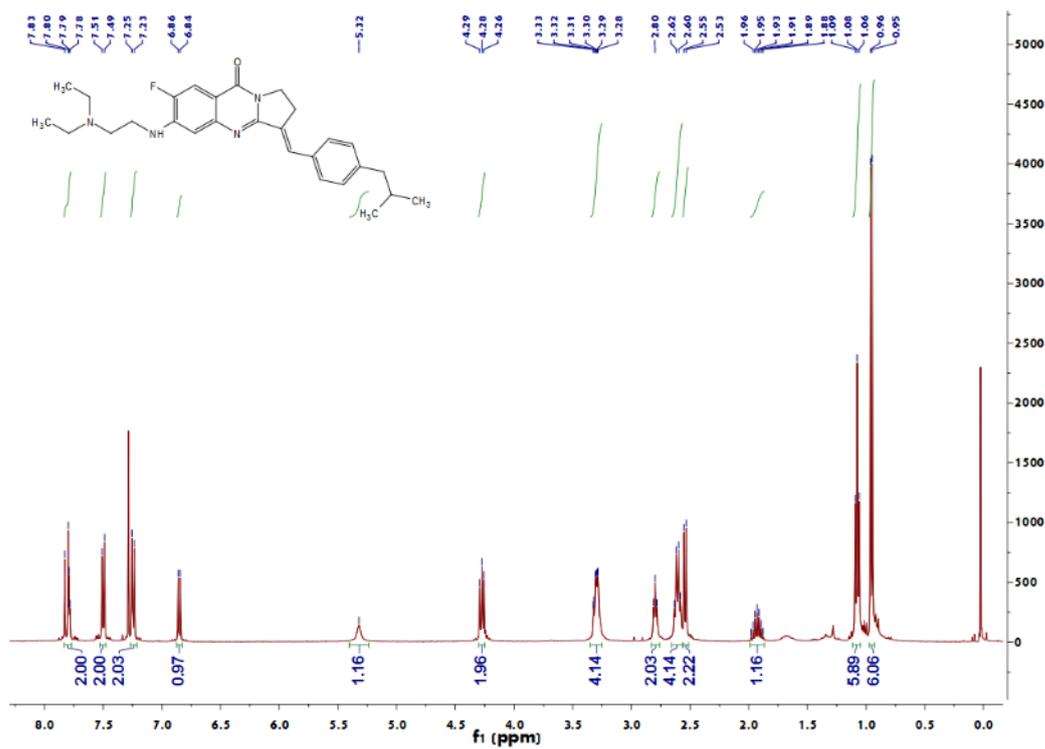


C27 H33 N4 O F [M+H]⁺ : Predicted region for 449.2711 m/z

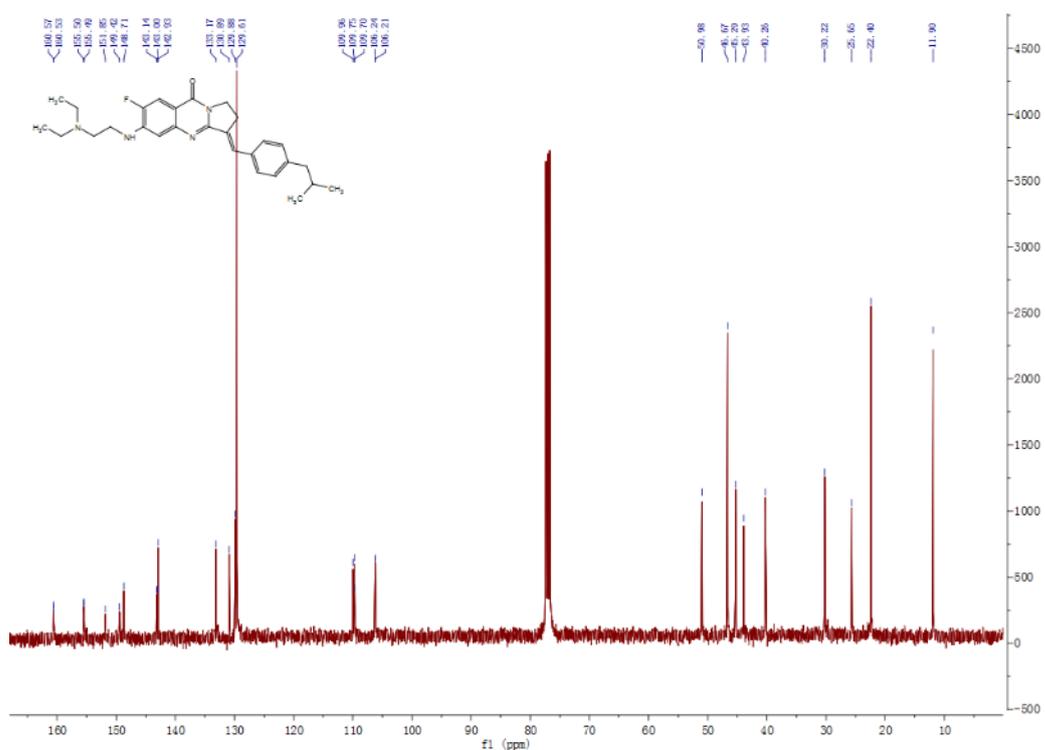


Rank	Score	Formula (M)	Ion	Mess. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Isot	DBE
6	87.82	C27 H33 N4 O F	[M+H] ⁺	449.2702	449.2711	-0.9	-2.00	90.07	13.0

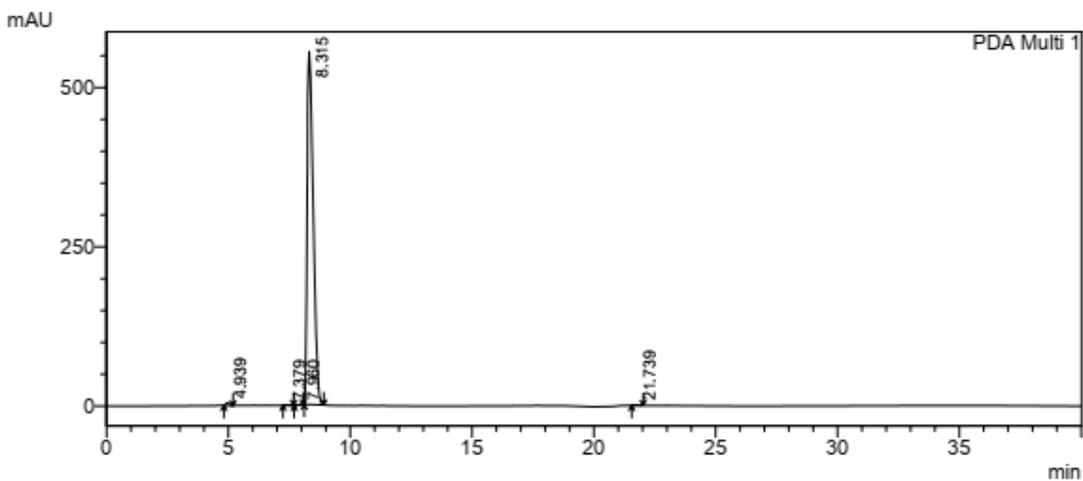
HRMS spectrum of 7f



¹H NMR spectrum of **8f**



¹³C NMR spectrum of **8f**



1 PDA Multi 1/254nm 4nm

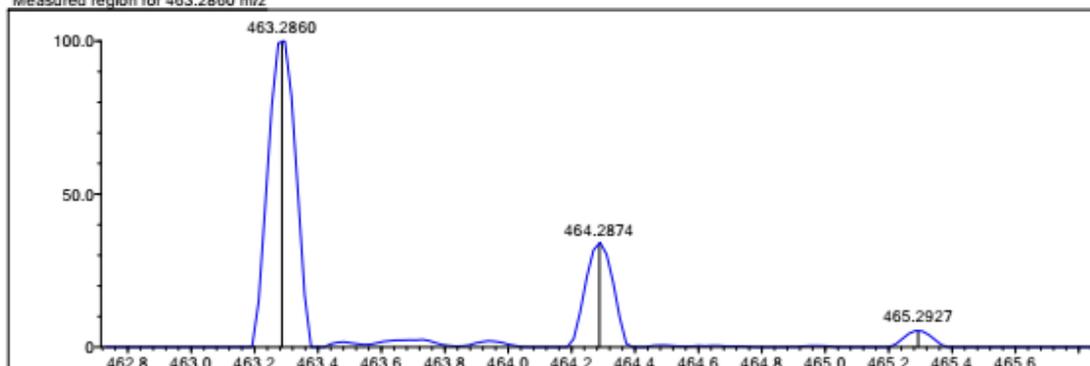
PeakTable

PDA Ch1 254nm 4nm

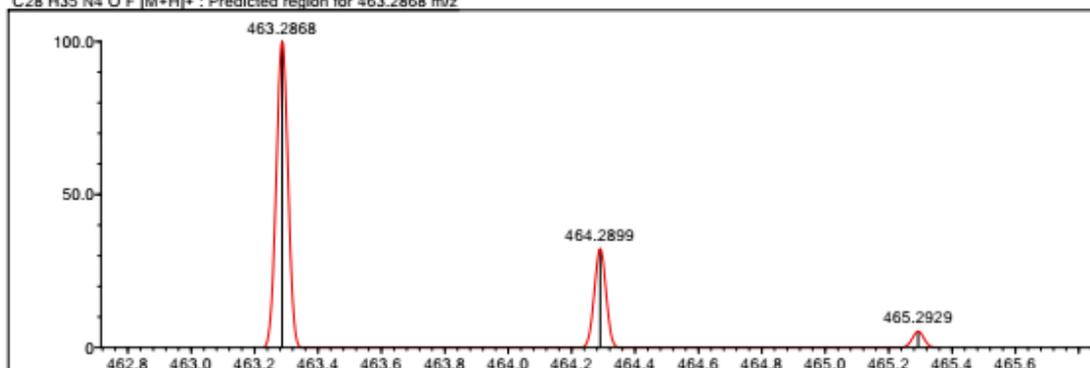
Peak#	Ret. Time	Area	Height	Area %	Height %
1	4.939	50320	4127	0.523	0.736
2	7.379	8862	626	0.092	0.112
3	7.960	11292	1423	0.117	0.254
4	8.315	9539199	554105	99.207	98.836
5	21.739	5741	349	0.060	0.062
Total		9615413	560630	100.000	100.000

HPLC analysis of **8f**

Measured region for 463.2860 m/z

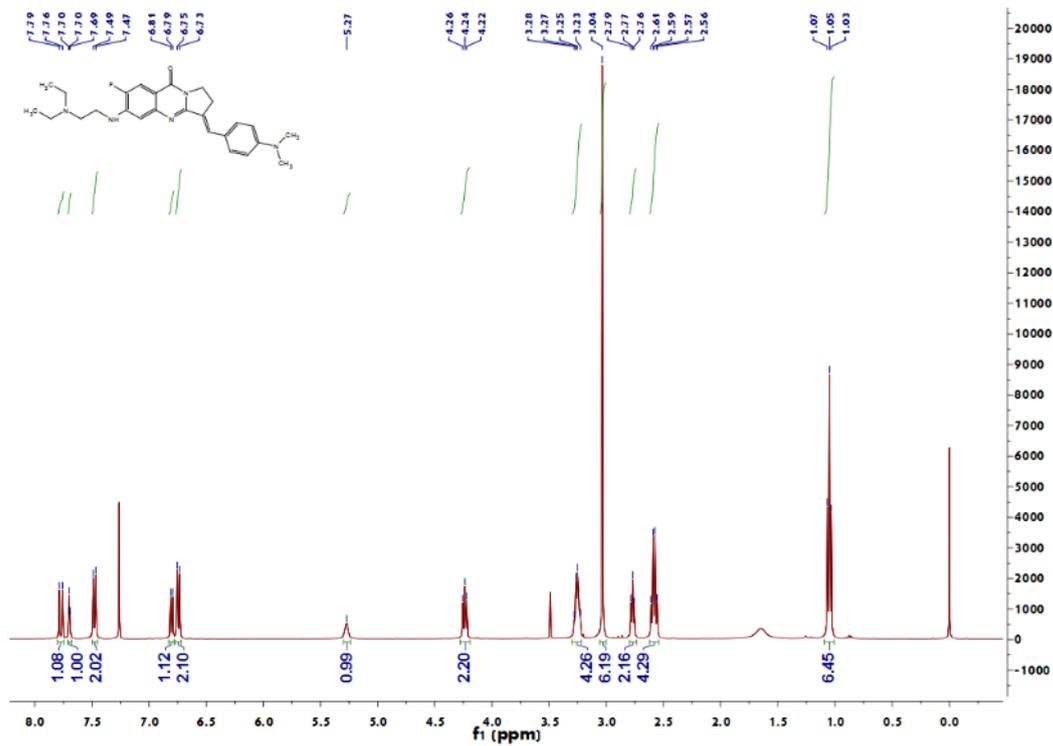


C28 H35 N4 O F [M+H]⁺ : Predicted region for 463.2868 m/z

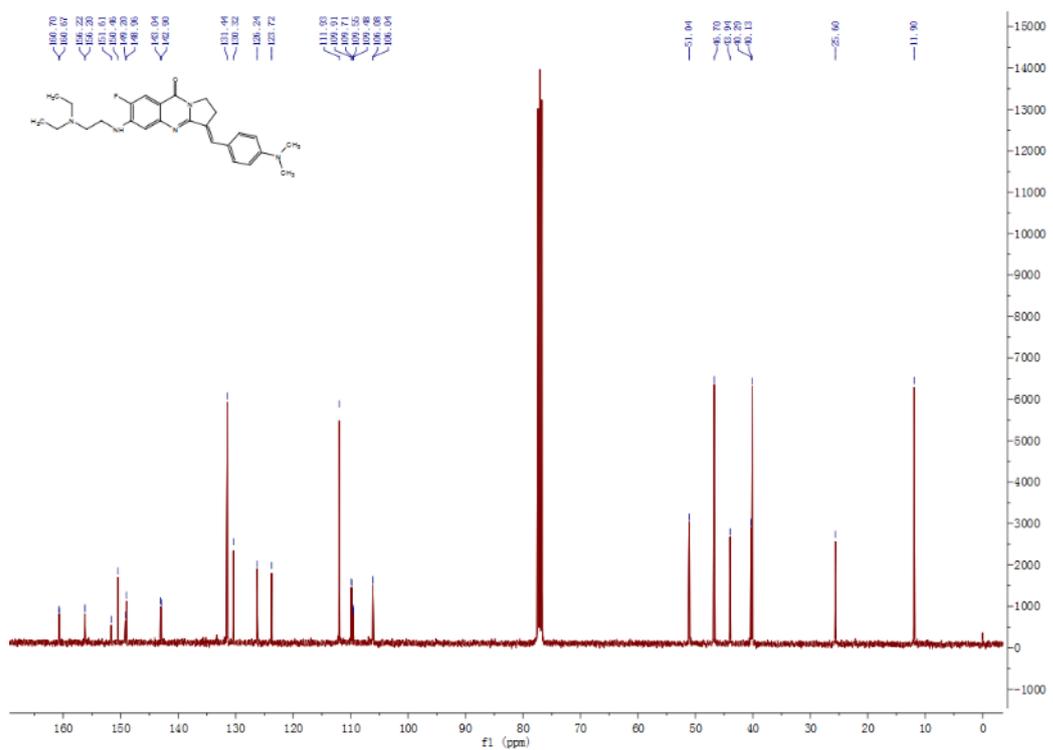


Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
2	88.58	C28 H35 N4 O F	[M+H] ⁺	463.2860	463.2868	-0.8	-1.73	90.23	13.0

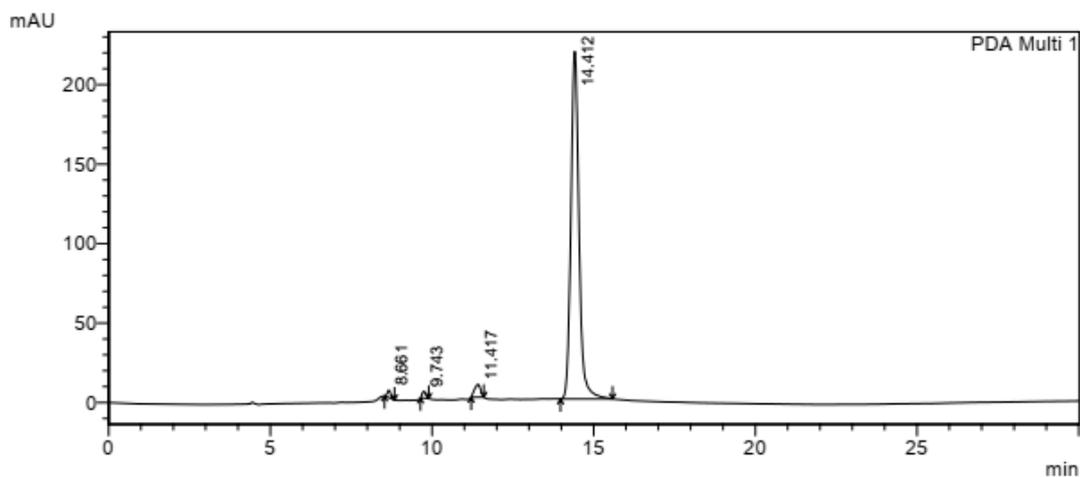
HRMS spectrum of **8f**



¹H NMR spectrum of **9f**



¹³C NMR spectrum of **9f**

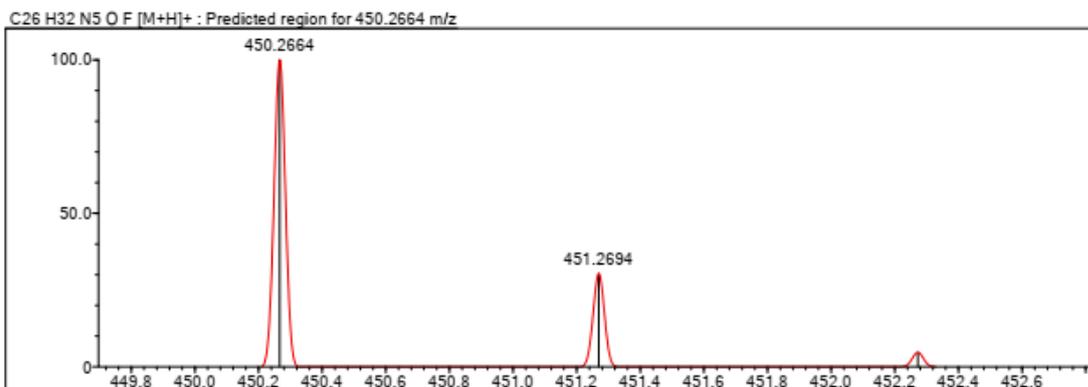
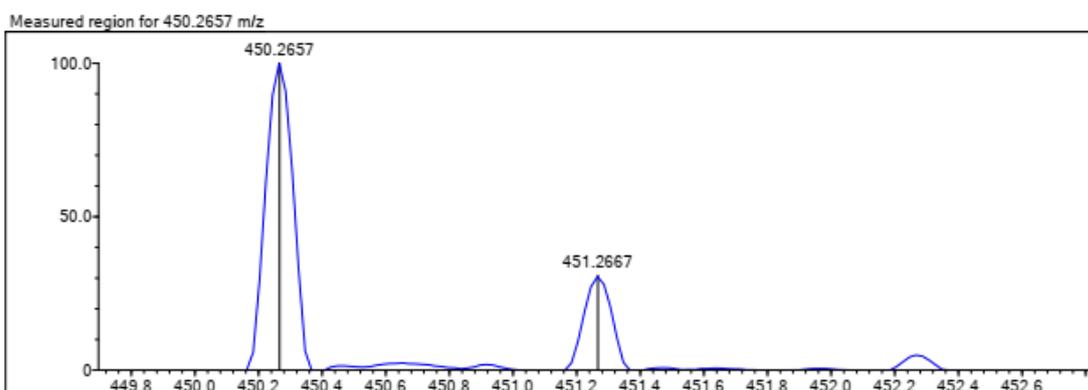


1 PDA Multi 1/254nm 4nm

PeakTable

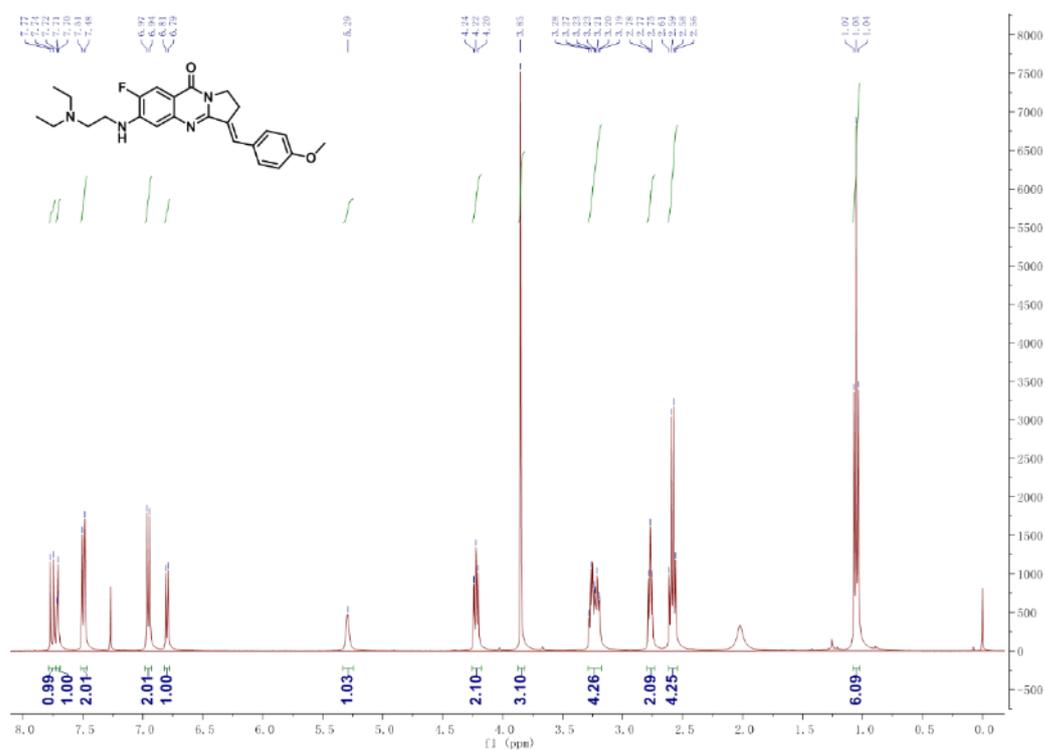
Peak#	Ret. Time	Area	Height	Area %	Height %
1	8.661	32967	4643	0.832	1.972
2	9.743	34067	4248	0.860	1.804
3	11.417	104440	7998	2.636	3.397
4	14.412	3790498	218566	95.672	92.827
Total		3961972	235454	100.000	100.000

HPLC analysis of **9f**

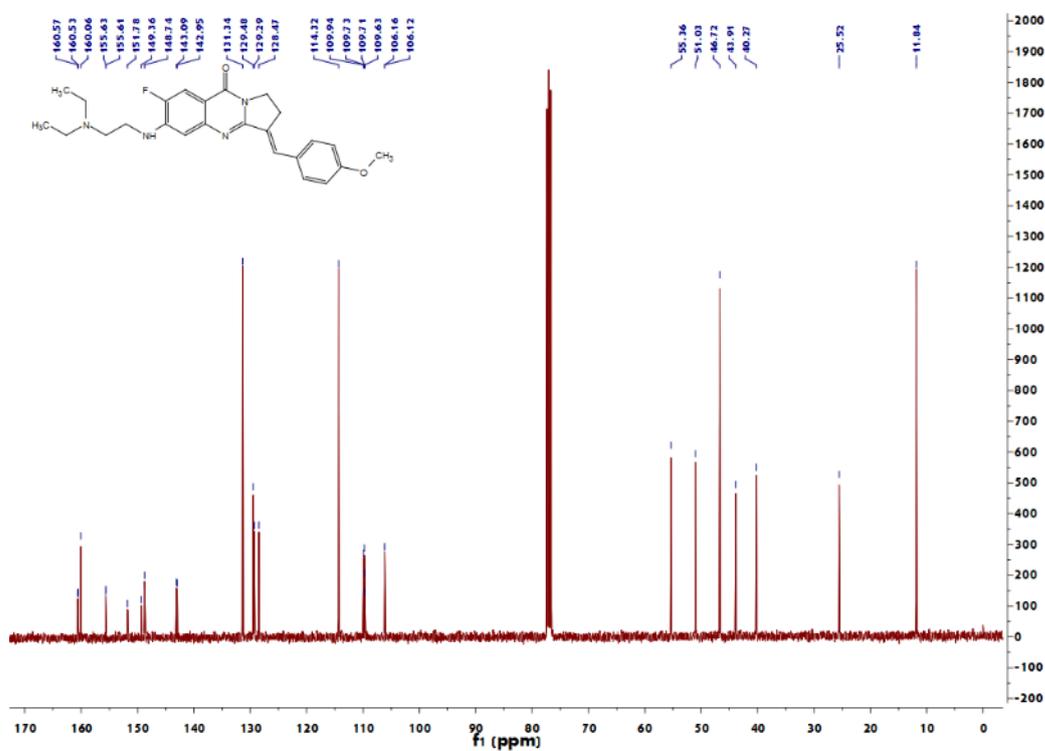


Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	90.40	C26 H32 N5 O F	[M+H] ⁺	450.2657	450.2664	-0.7	-1.55	91.66	13.0

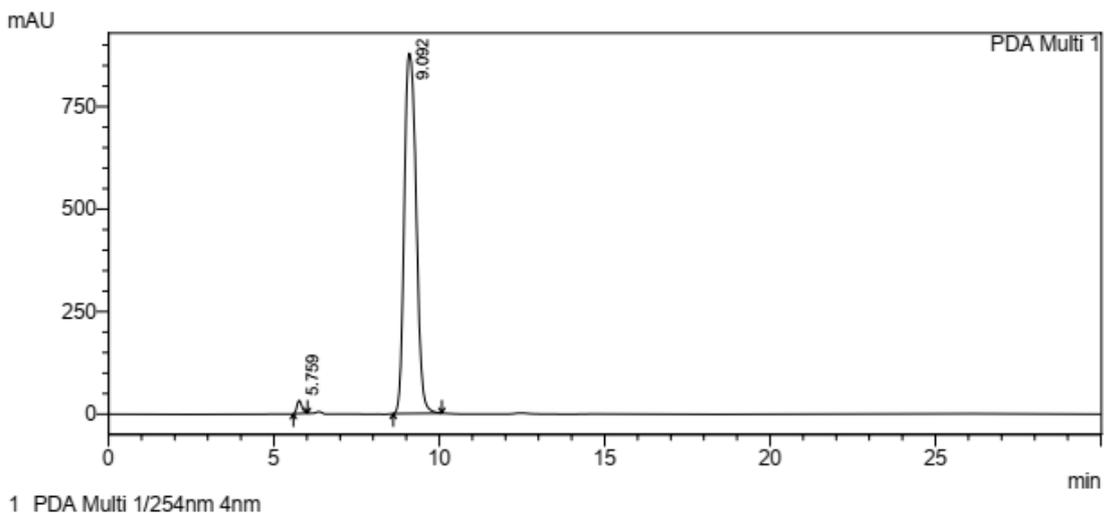
HRMS spectrum of **9f**



¹H NMR spectrum of 10f



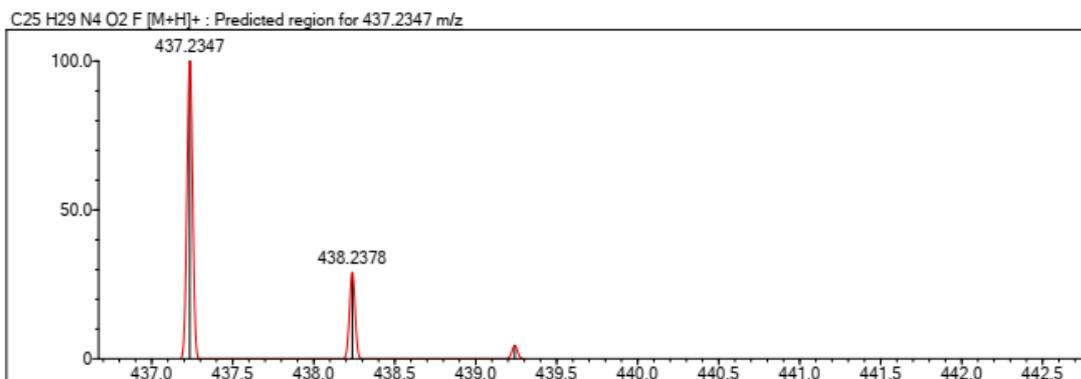
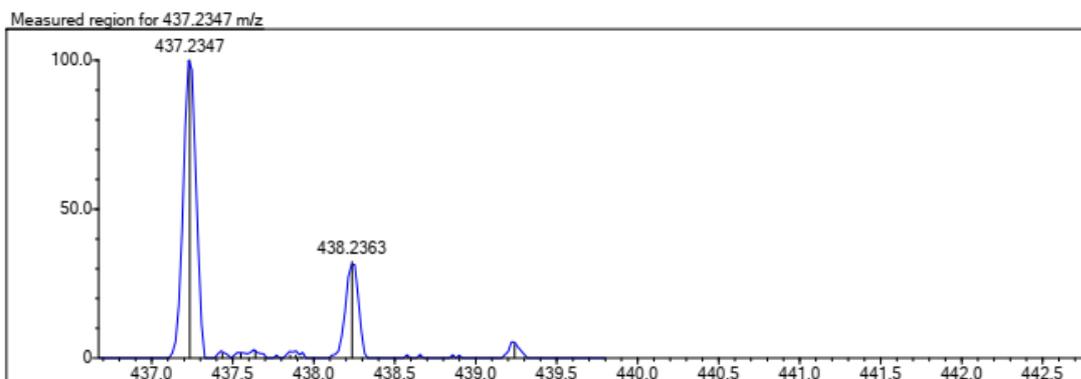
¹³C NMR spectrum of 10f



PeakTable

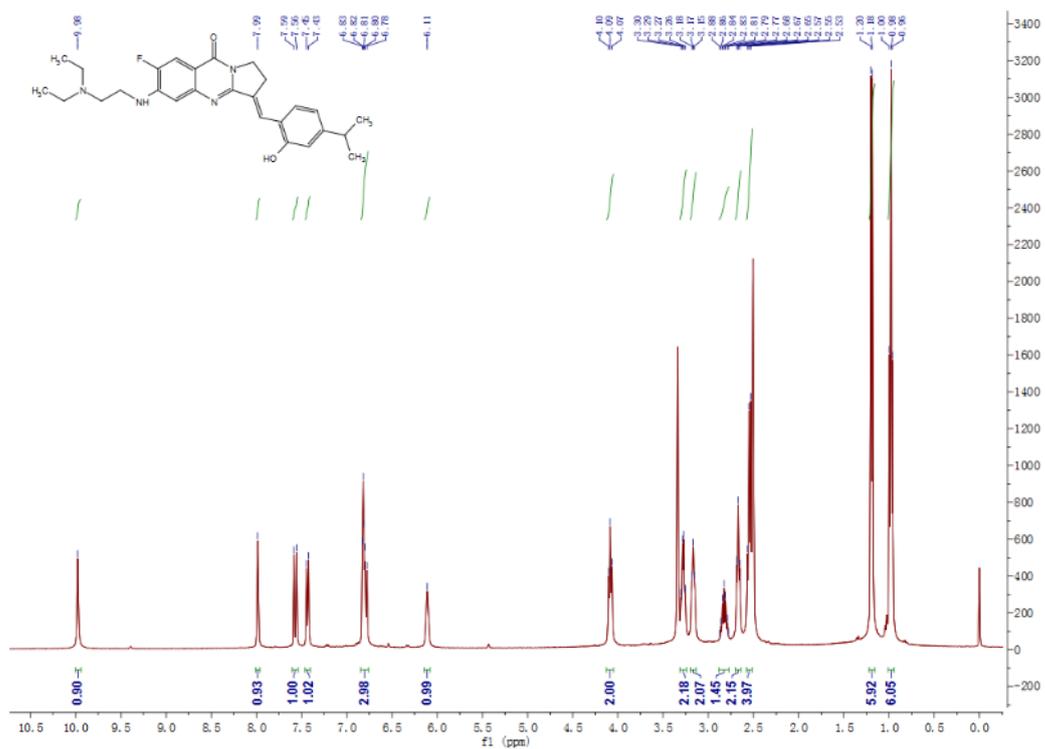
Peak#	Ret. Time	Area	Height	Area %	Height %
1	5.759	330409	31599	1.485	3.472
2	9.092	21919063	878540	98.515	96.528
Total		22249471	910139	100.000	100.000

HPLC analysis of **10f**

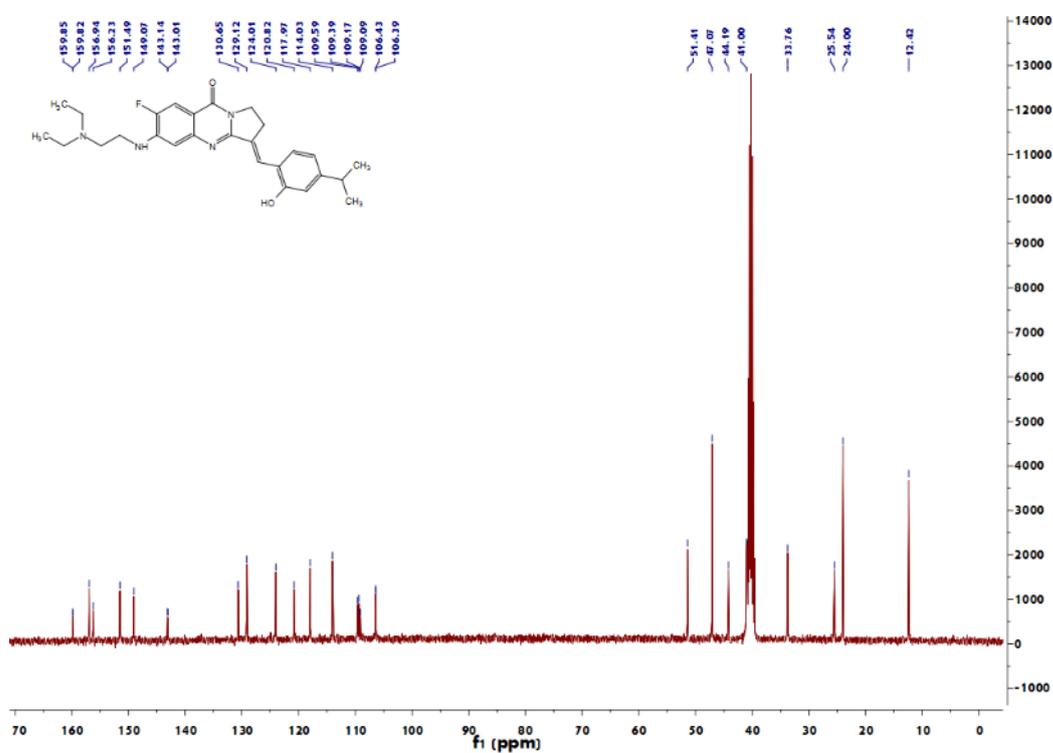


Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	82.60	C25 H29 N4 O2 F	[M+H] ⁺	437.2347	437.2347	-0.0	0.00	82.60	13.0

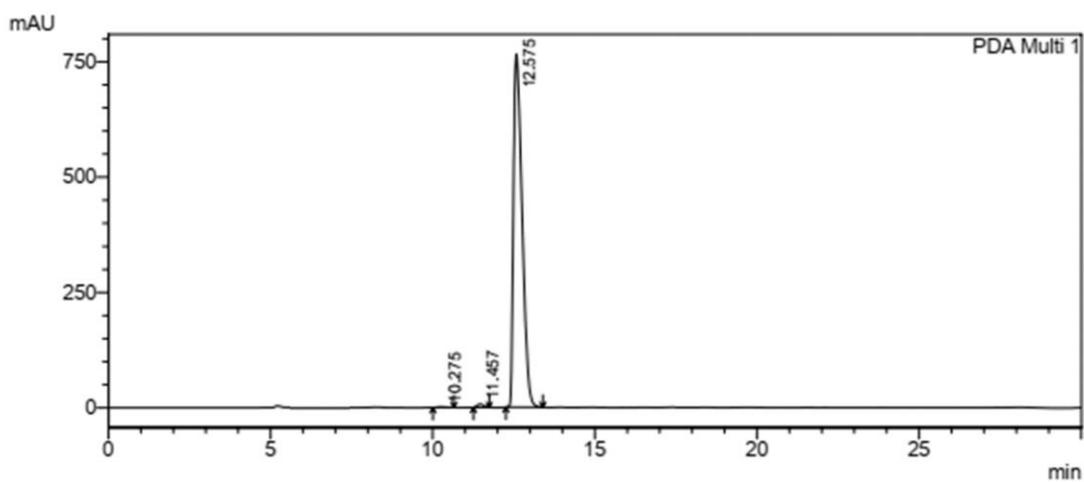
HRMS spectrum of **10f**



¹H NMR spectrum of 11f



¹³C NMR spectrum of 11f



1 PDA Multi 1/254nm 4nm

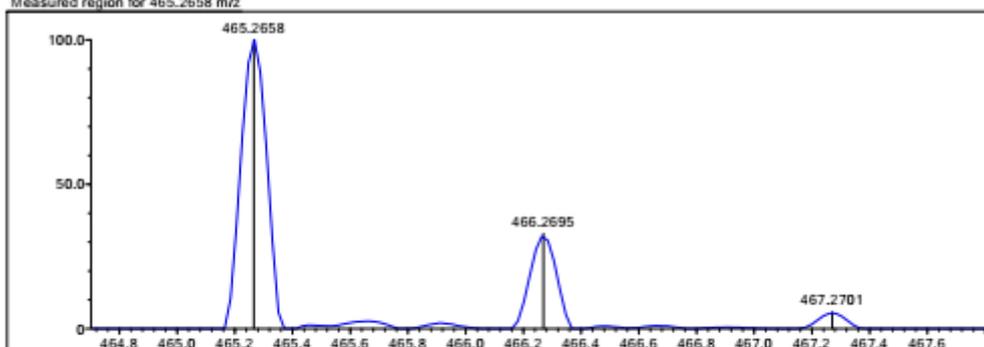
PeakTable

PDA Chl 254nm 4nm

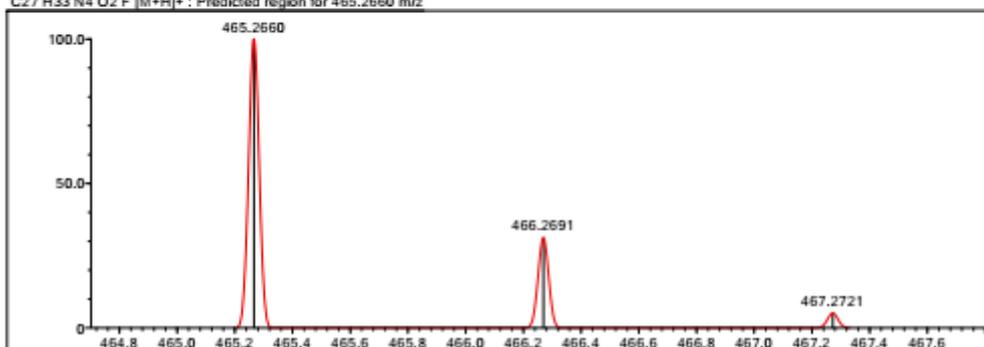
Peak#	Ret. Time	Area	Height	Area %	Height %
1	10.275	34241	1523	0.242	0.197
2	11.457	98974	7149	0.699	0.924
3	12.575	14024382	765276	99.059	98.880
Total		14157597	773948	100.000	100.000

HPLC analysis of 11f

Measured region for 465.2658 m/z

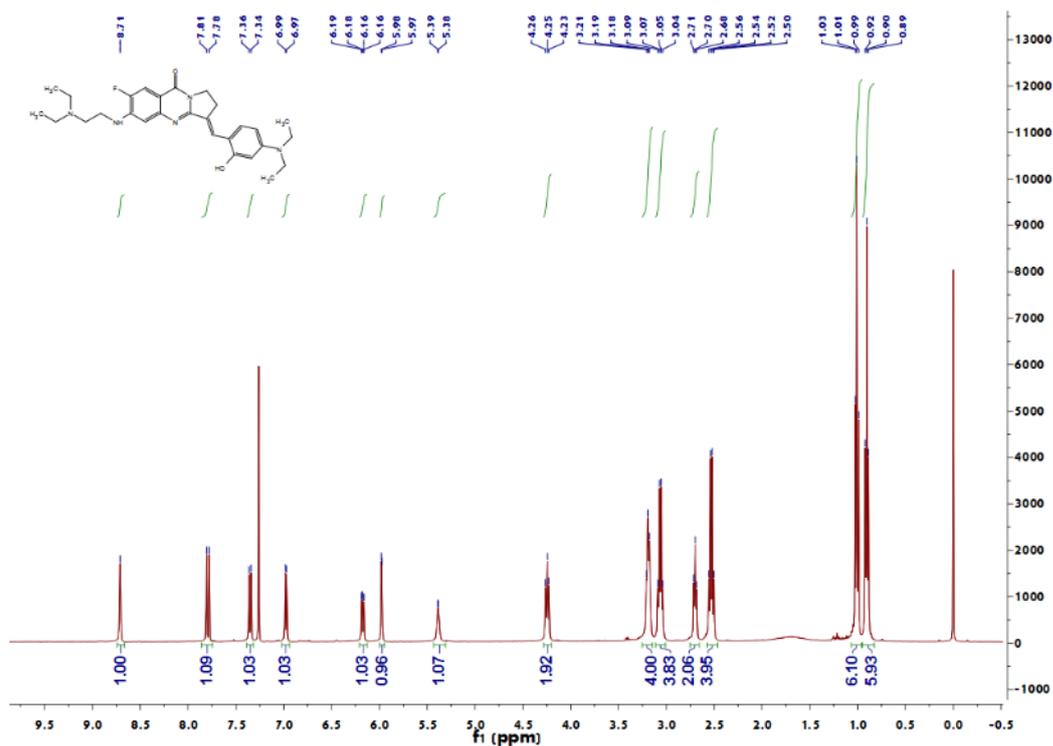


C27 H33 N4 O2 F [M+H]⁺ : Predicted region for 465.2660 m/z

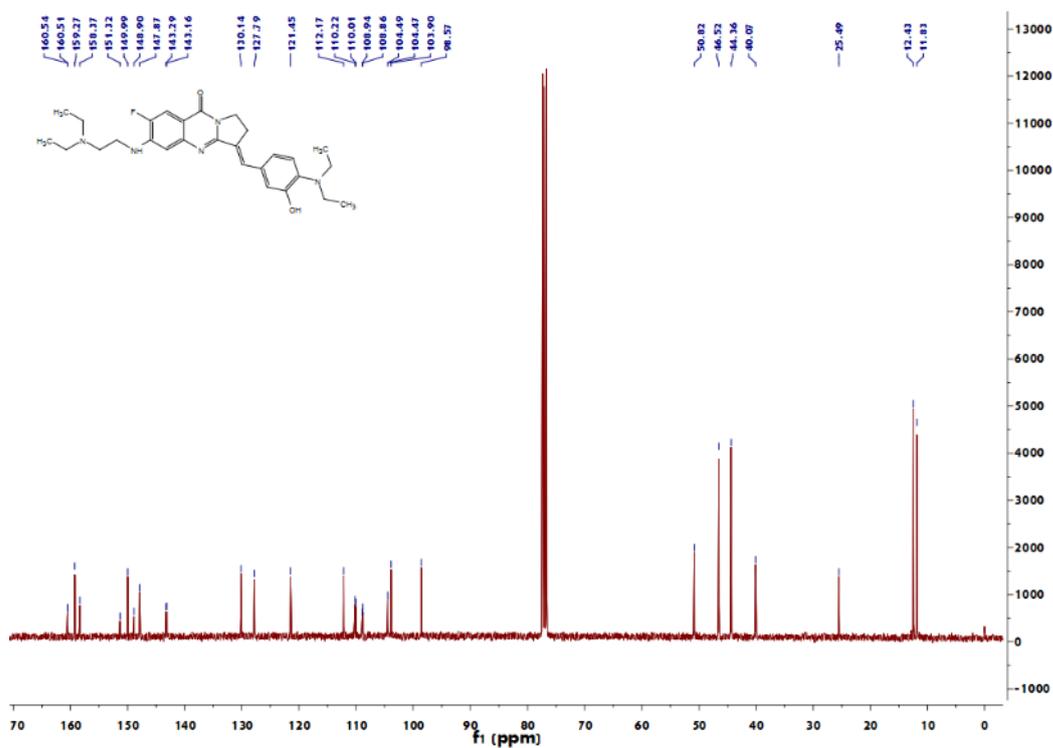


Rank	Score	Formula (M)	Ion	Mass. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	86.09	C27 H33 N4 O2 F	[M+H] ⁺	465.2658	465.2660	-0.2	-0.43	86.09	13.0

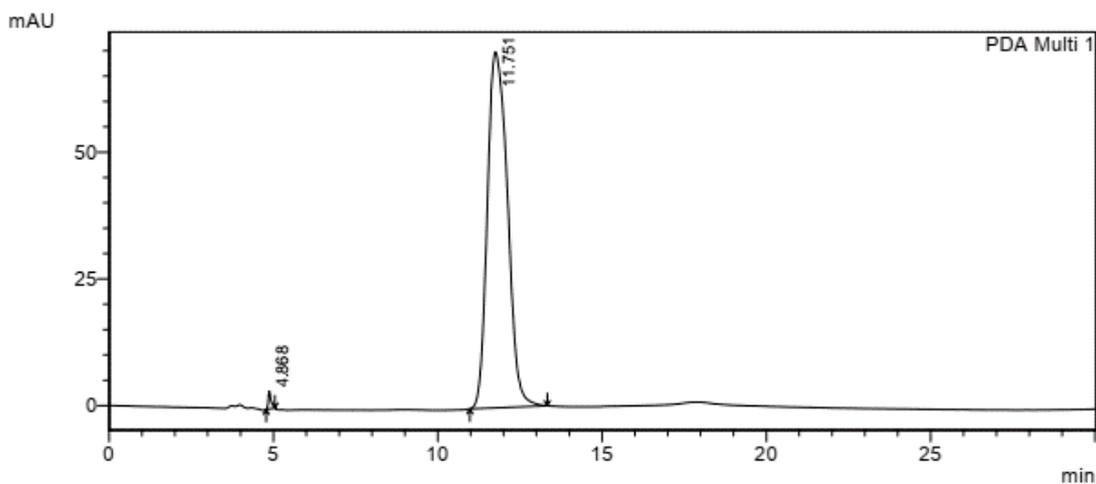
HRMS spectrum of 11f



¹H NMR spectrum of 12f



¹³C NMR spectrum of 12f



1 PDA Multi 1/254nm 4nm

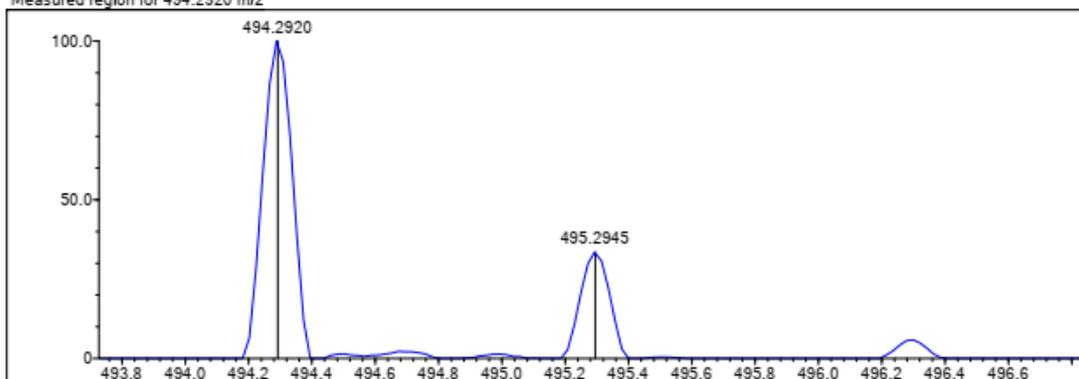
PeakTable

PDA Ch1 254nm 4nm

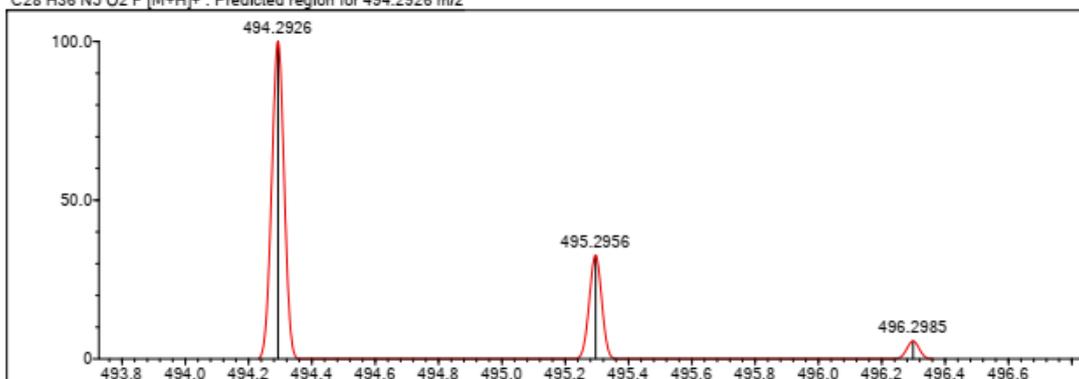
Peak#	Ret. Time	Area	Height	Area %	Height %
1	4.868	21150	3462	0.703	4.699
2	11.751	2989252	70212	99.297	95.301
Total		3010402	73674	100.000	100.000

HPLC analysis of **12f**

Measured region for 494.2920 m/z

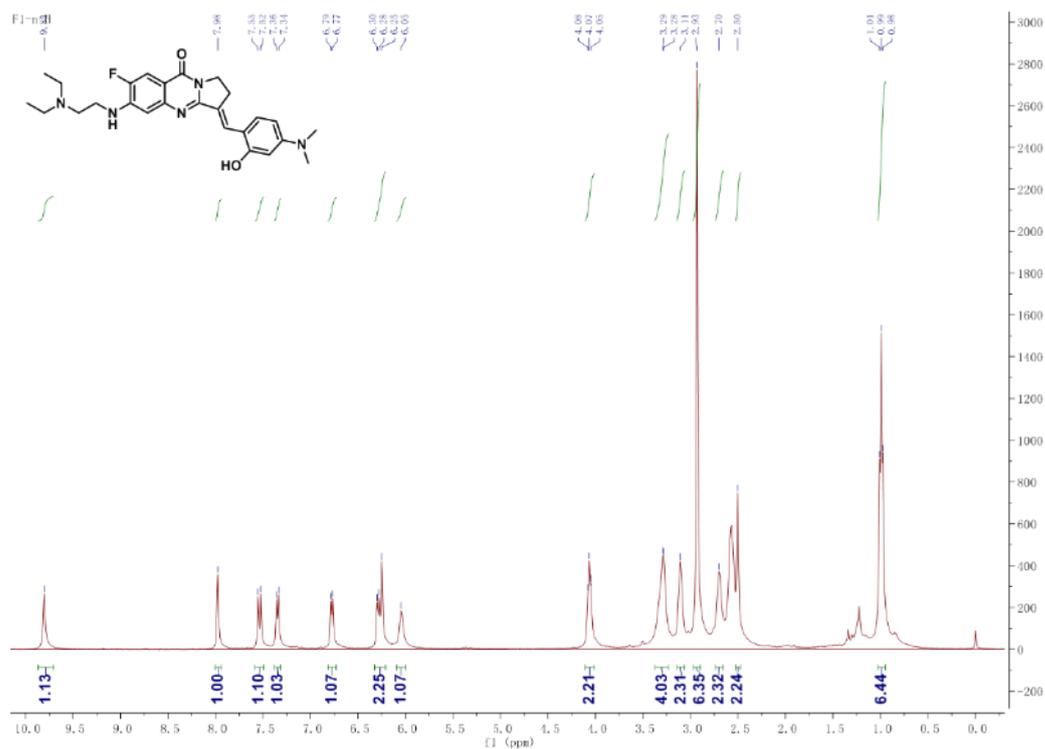


C28 H36 N5 O2 F [M+H]⁺ : Predicted region for 494.2926 m/z

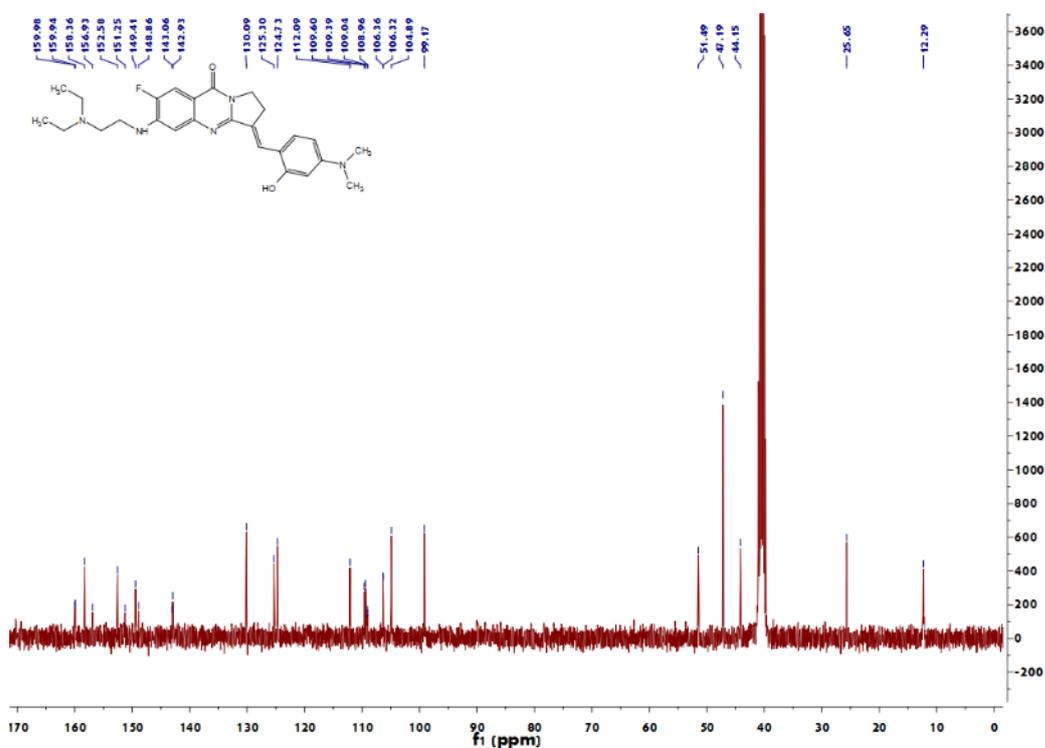


Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Isot	DBE
6	79.33	C28 H36 N5 O2 F	[M+H] ⁺	494.2920	494.2926	-0.6	-1.21	79.75	13.0

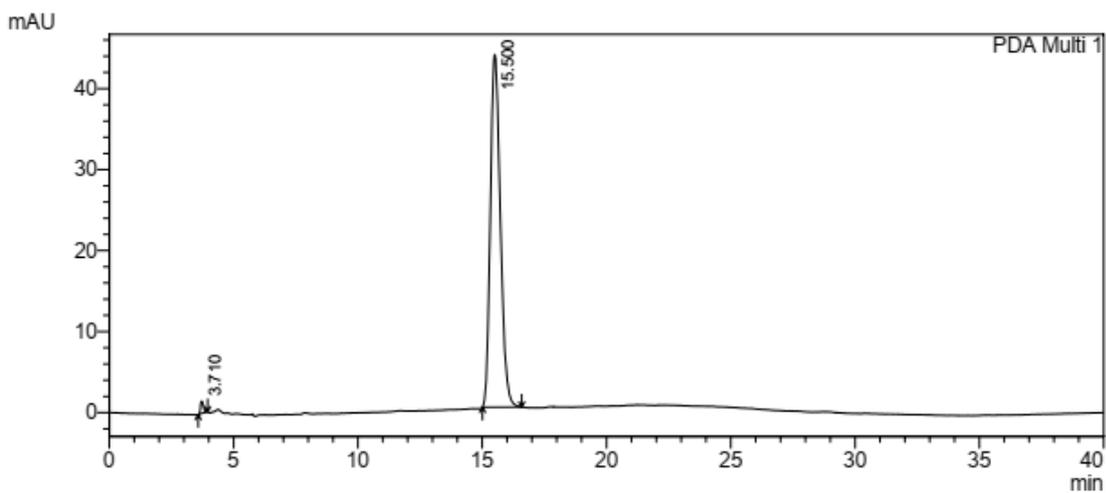
HRMS spectrum of **12f**



¹H NMR spectrum of 13f



¹³C NMR spectrum of 13f



1 PDA Multi 1/254nm 4nm

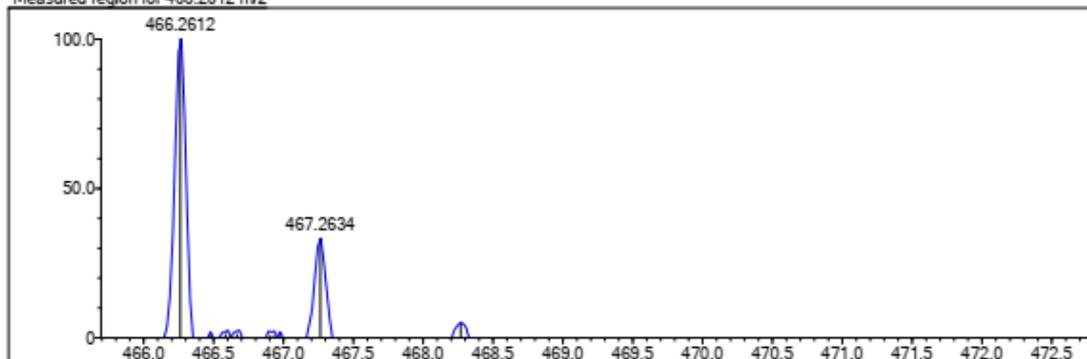
PeakTable

PDA Ch1 254nm 4nm

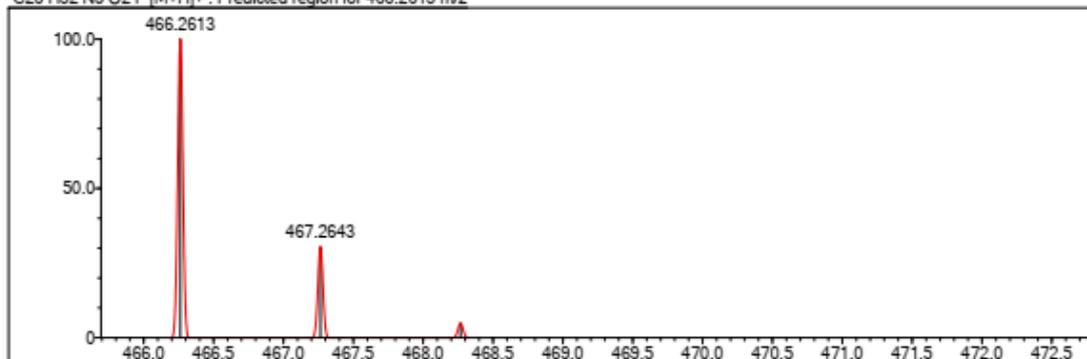
Peak#	Ret. Time	Area	Height	Area %	Height %
1	3.710	12672	1524	1.033	3.381
2	15.500	1214374	43542	98.967	96.619
Total		1227046	45065	100.000	100.000

HPLC analysis of **13f**

Measured region for 466.2612 m/z

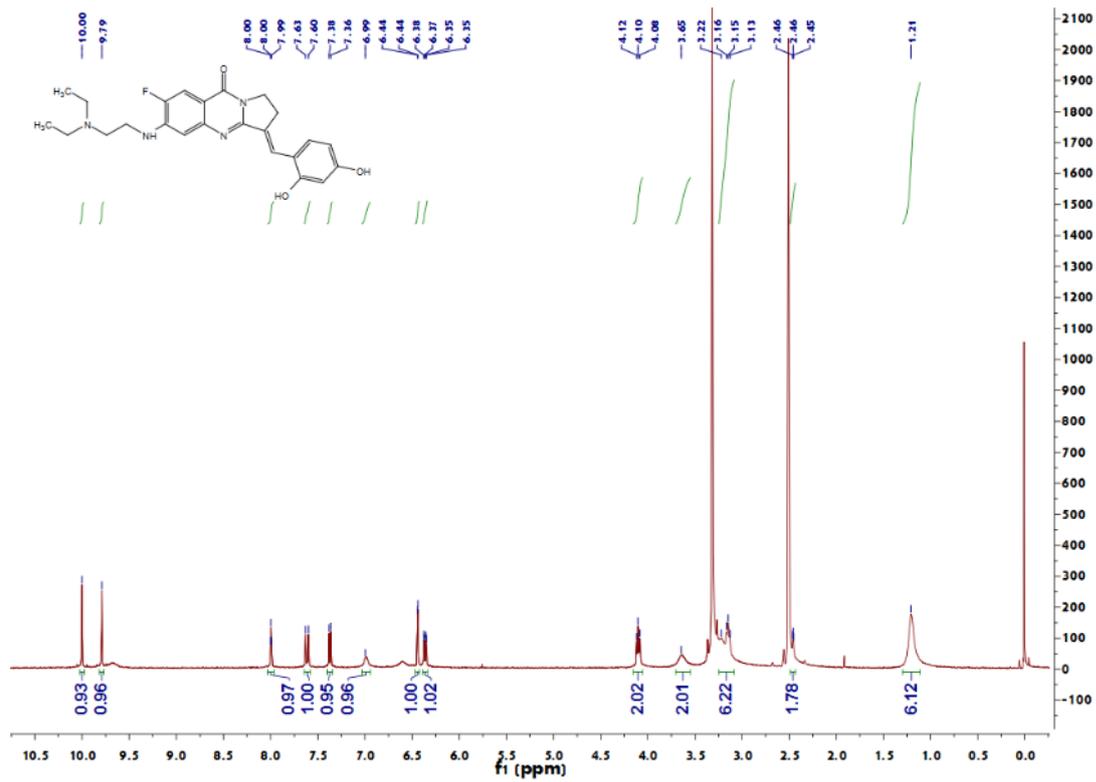


C26 H32 N5 O2 F [M+H]⁺ : Predicted region for 466.2613 m/z

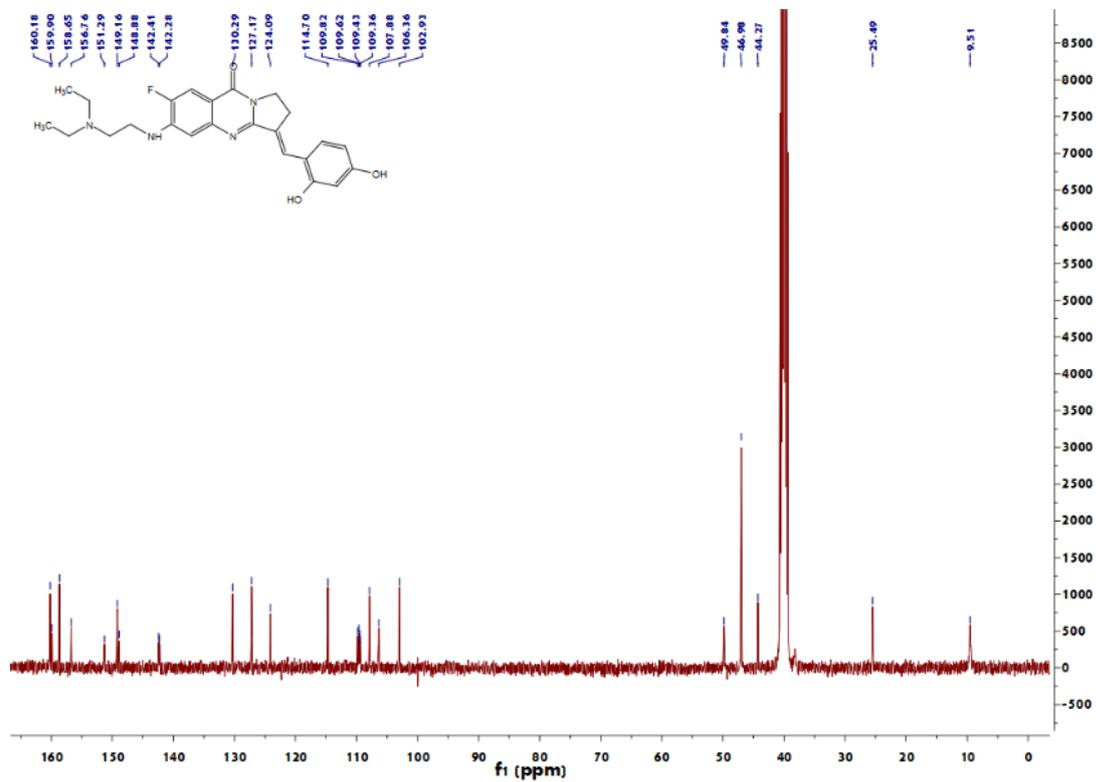


Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	87.71	C26 H32 N5 O2 F	[M+H] ⁺	466.2612	466.2613	-0.1	-0.21	87.71	13.0

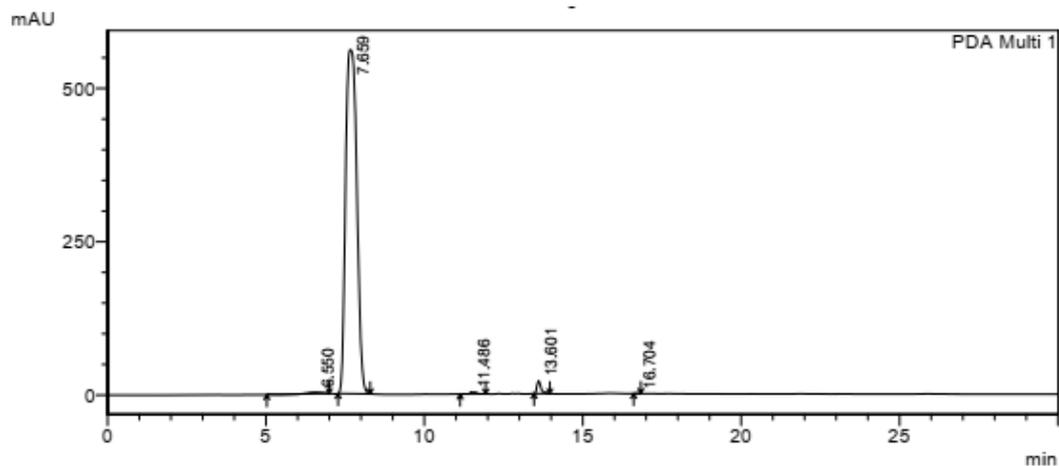
HRMS spectrum of **13f**



¹H NMR spectrum of 14f



¹³C NMR spectrum of 14f



1 PDA Multi 1/254nm 4nm

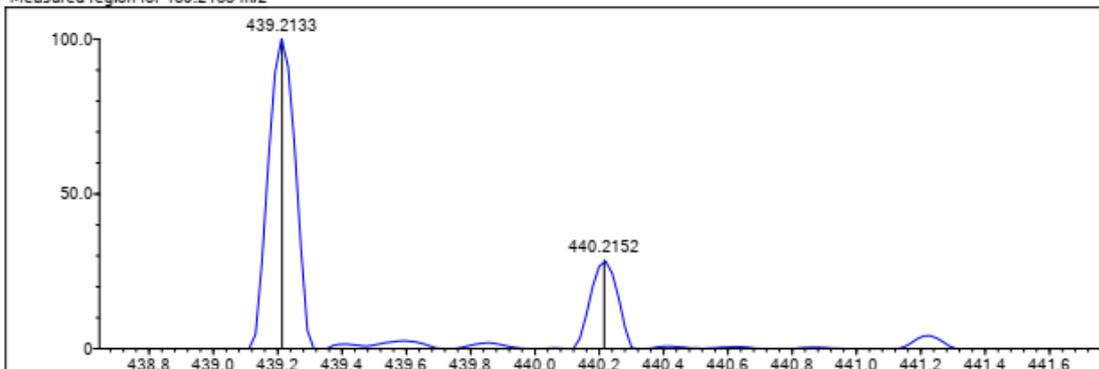
PeakTable

PDA Ch1 254nm 4nm

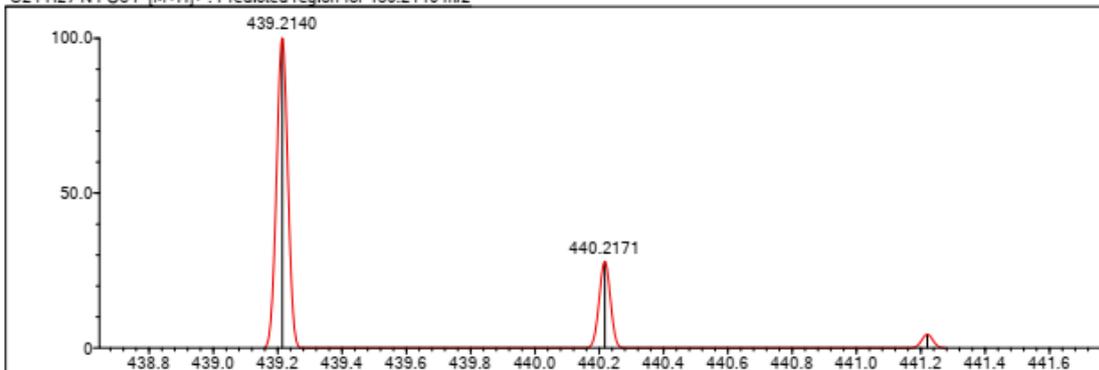
Peak#	Ret. Time	Area	Height	Area %	Height %
1	6.550	67123	2352	0.477	0.400
2	7.659	13781792	560595	97.837	95.457
3	11.486	50046	3296	0.355	0.361
4	13.601	184601	20658	1.310	3.518
5	16.704	2949	374	0.021	0.064
Total		14086511	587275	100.000	100.000

HPLC analysis of 14f

Measured region for 439.2133 m/z

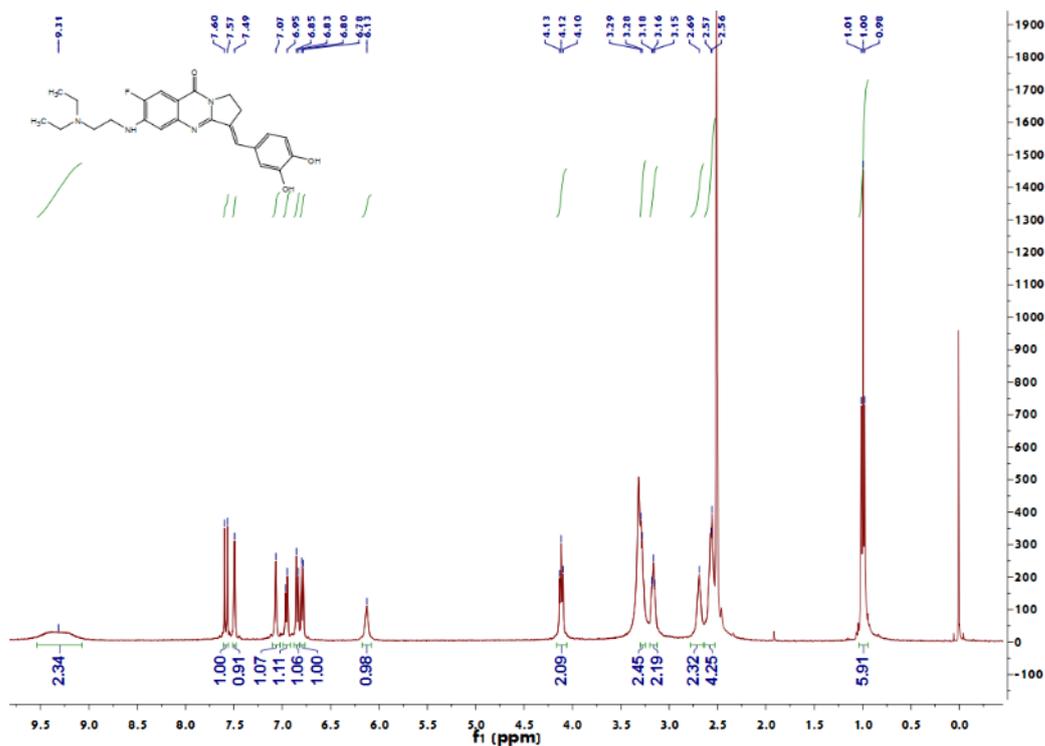


C24 H27 N4 O3 F [M+H]⁺ : Predicted region for 439.2140 m/z

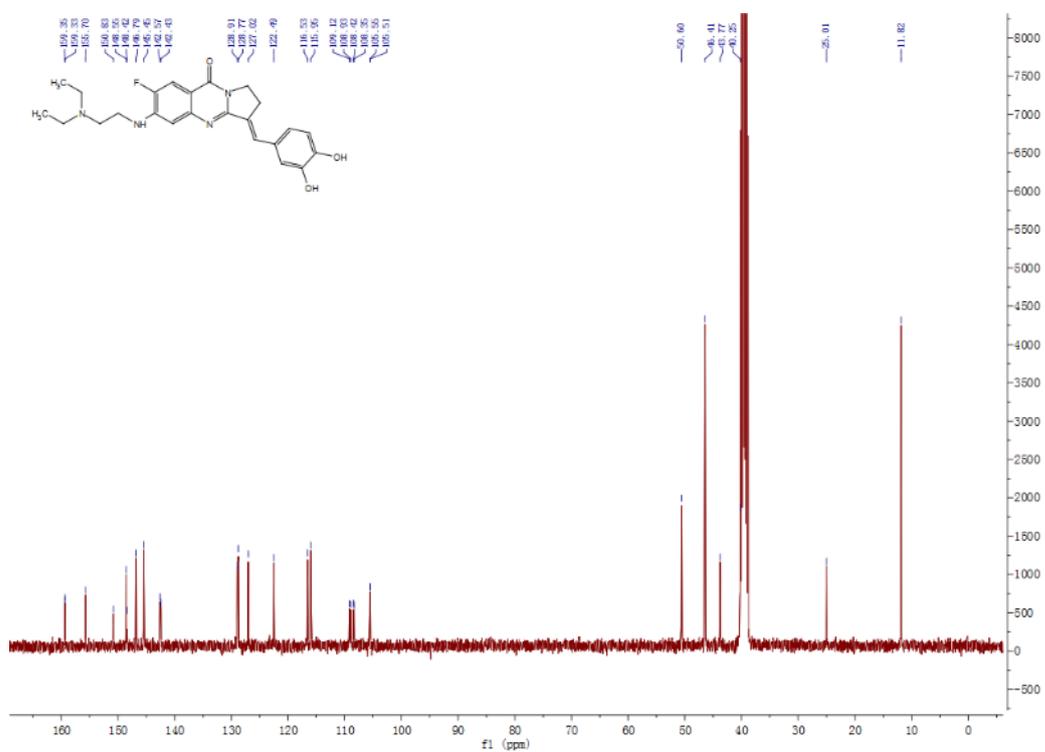


Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Isot	DBE
2	88.72	C24 H27 N4 O3 F	[M+H] ⁺	439.2133	439.2140	-0.7	-1.59	90.05	13.0

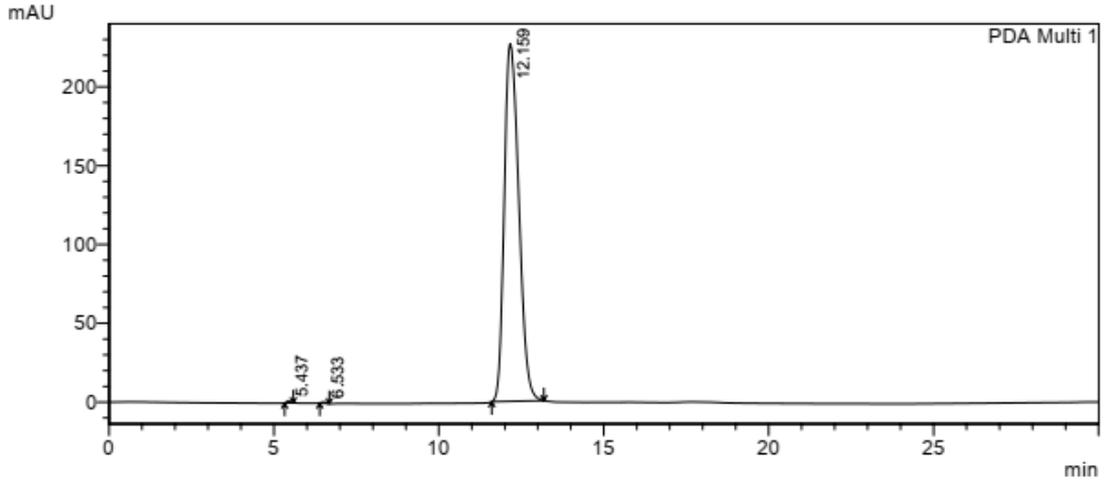
HRMS spectrum of 14f



¹H NMR spectrum of 15f



¹³C NMR spectrum of 15f



1 PDA Multi 1/254nm 4nm

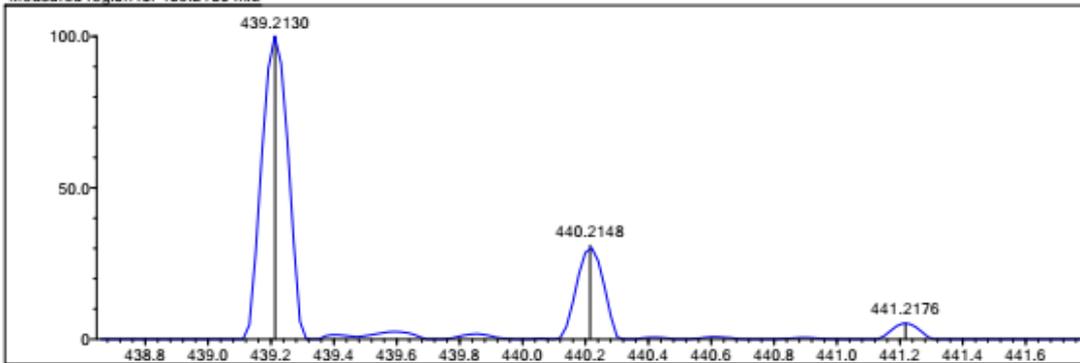
PeakTable

PDA Ch1 254nm 4nm

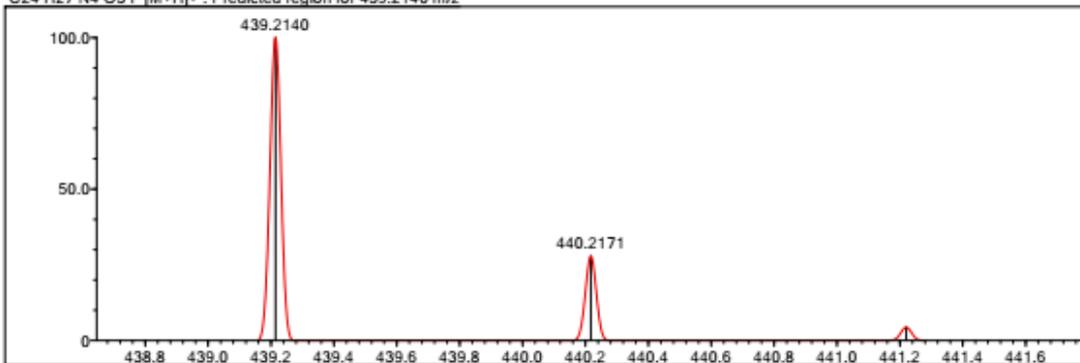
Peak#	Ret. Time	Area	Height	Area %	Height %
1	5.437	10604	1240	0.152	0.342
2	6.533	7260	808	0.104	0.353
3	12.159	6961523	226770	99.744	99.105
Total		6979387	228818	100.000	100.000

HPLC analysis of 15f

Measured region for 439.2130 m/z

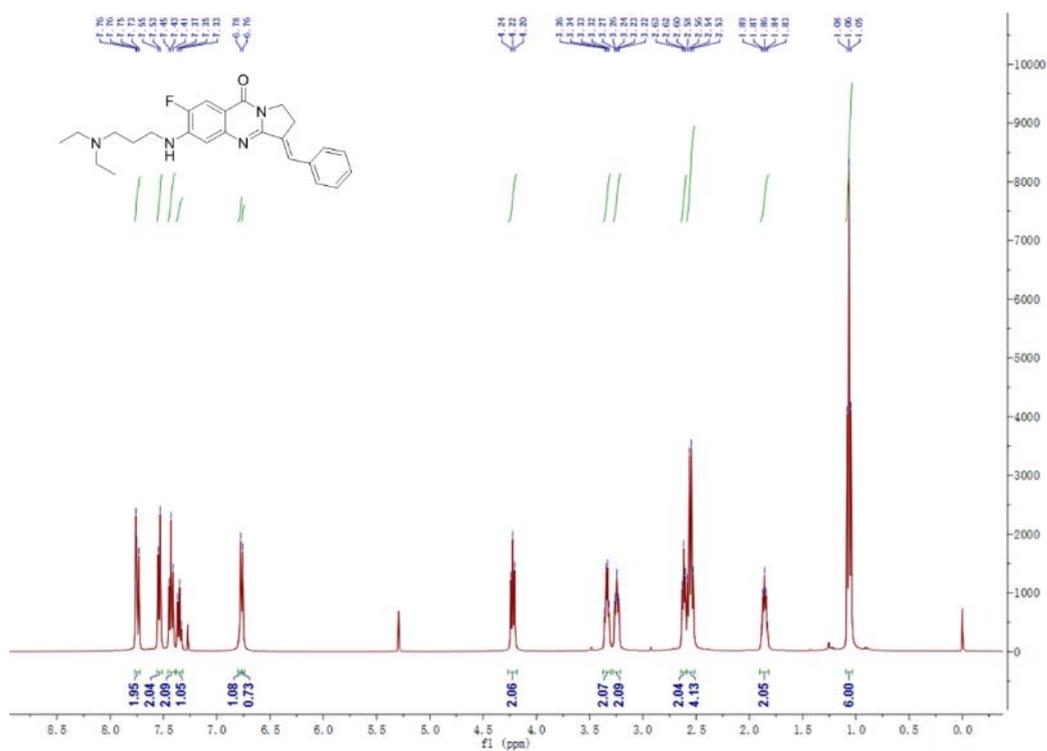


C24 H27 N4 O3 F [M+H]⁺ : Predicted region for 439.2140 m/z

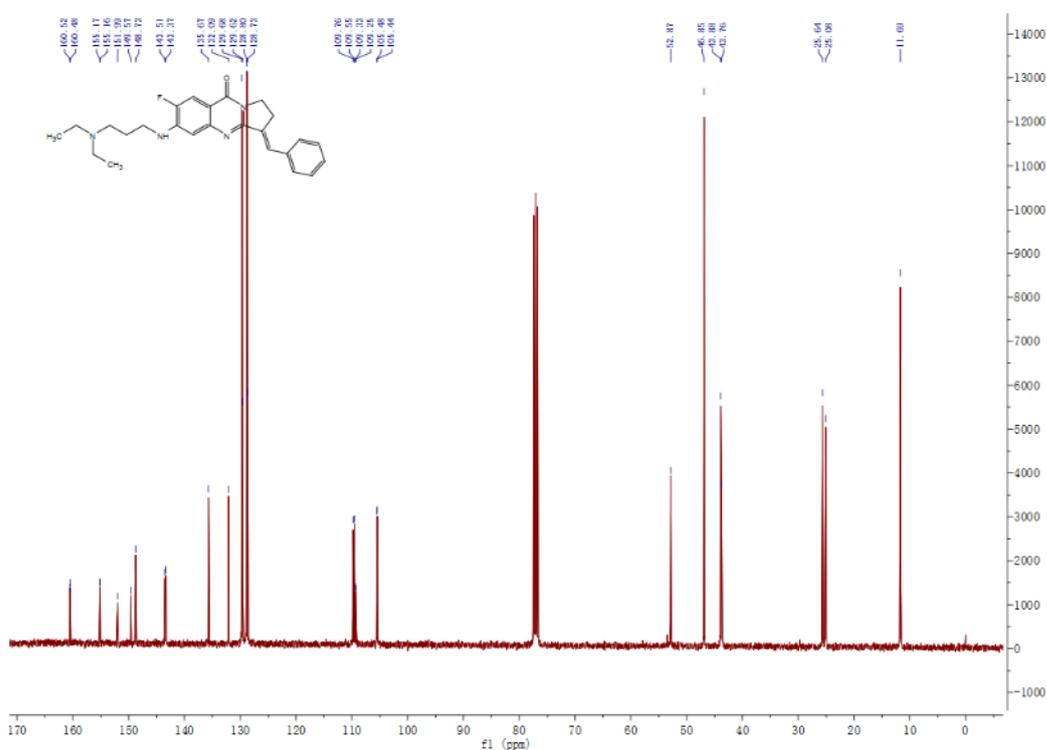


Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Isot	DBE
2	84.94	C24 H27 N4 O3 F	[M+H] ⁺	439.2130	439.2140	-1.0	-2.28	87.75	13.0

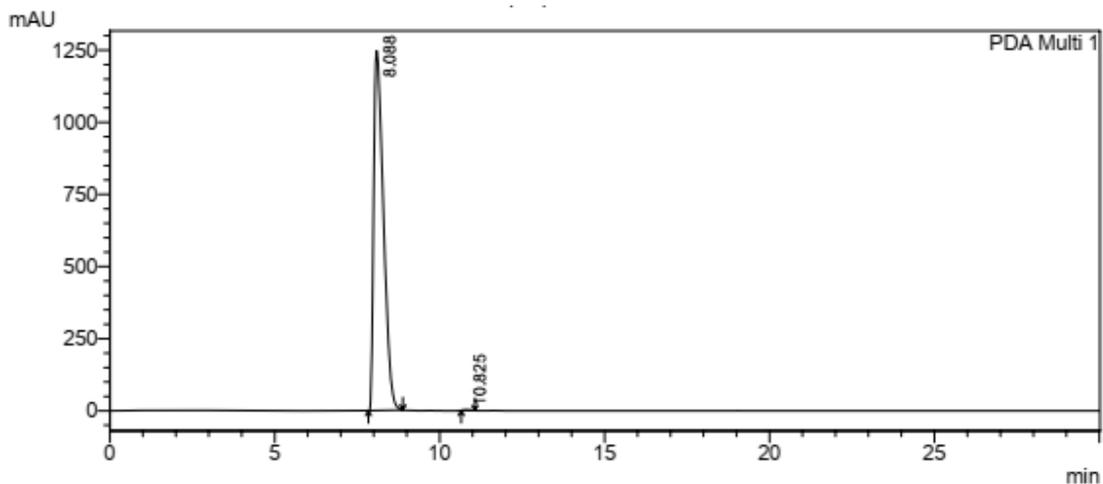
HRMS spectrum of 15f



¹H NMR spectrum of 16f



¹³C NMR spectrum of 16f

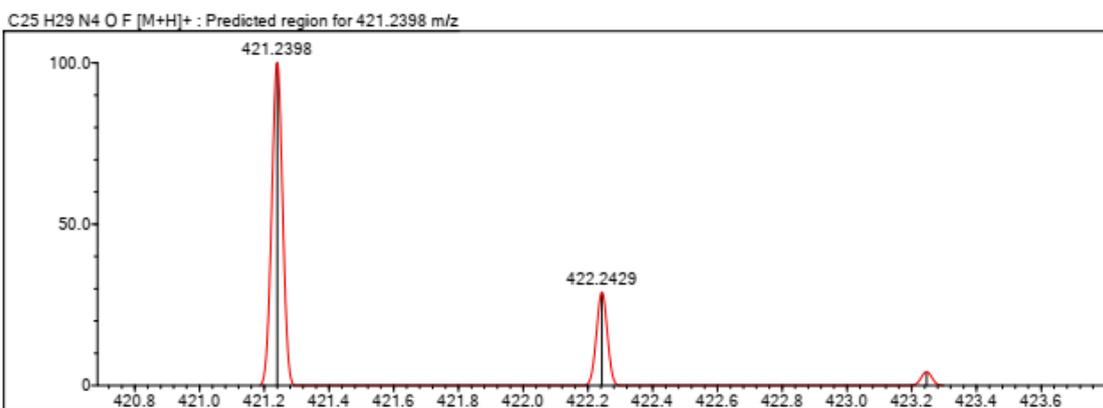
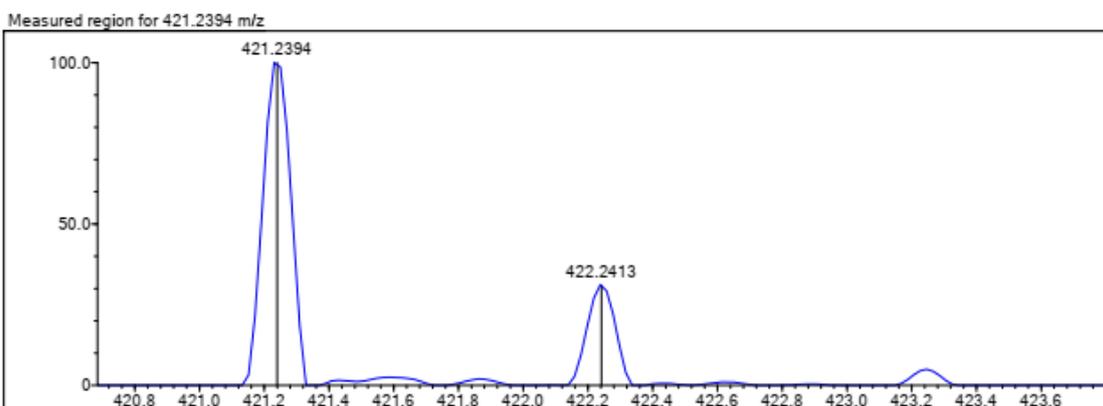


1 PDA Multi 1/254nm 4nm

PeakTable

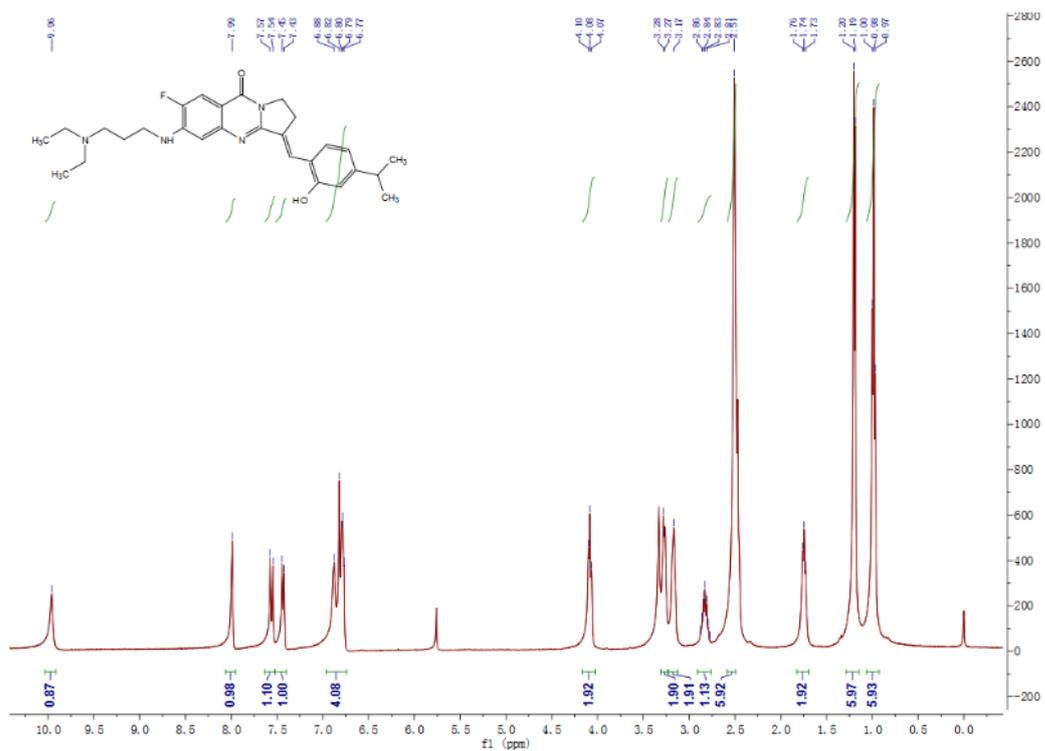
Peak#	Ret. Time	Area	Height	Area %	Height %
1	8.088	25649117	1246253	99.862	99.805
2	10.825	35415	2437	0.138	0.195
Total		25684532	1248690	100.000	100.000

HPLC analysis of 16f

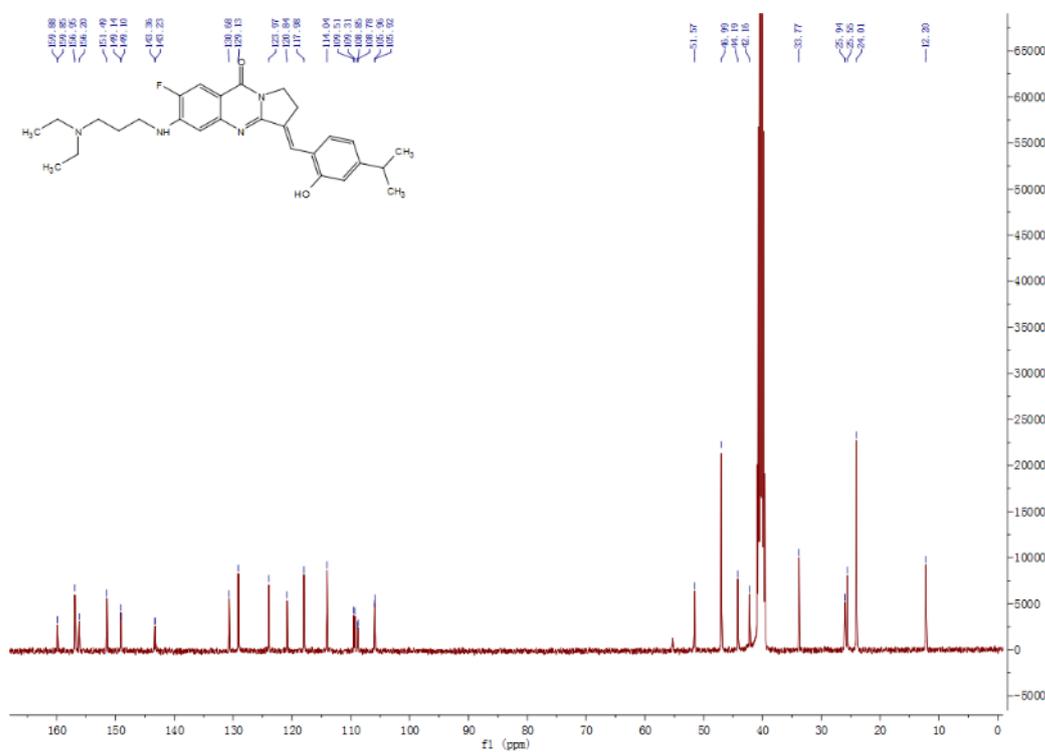


Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Isot	DBE
1	81.20	C25 H29 N4 O F	[M+H] ⁺	421.2394	421.2398	-0.4	-0.95	81.20	13.0

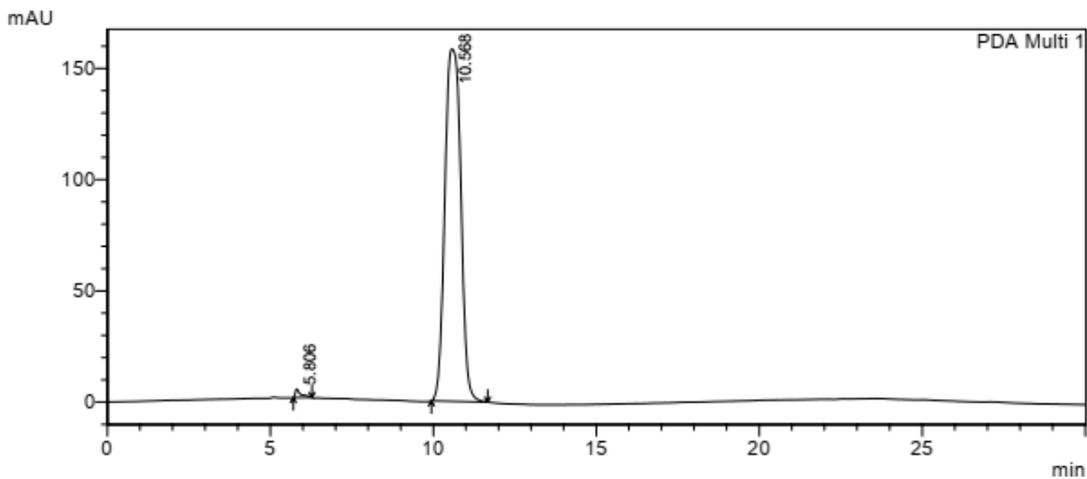
HRMS spectrum of 16f



¹H NMR spectrum of 17f



¹³C NMR spectrum of 17f



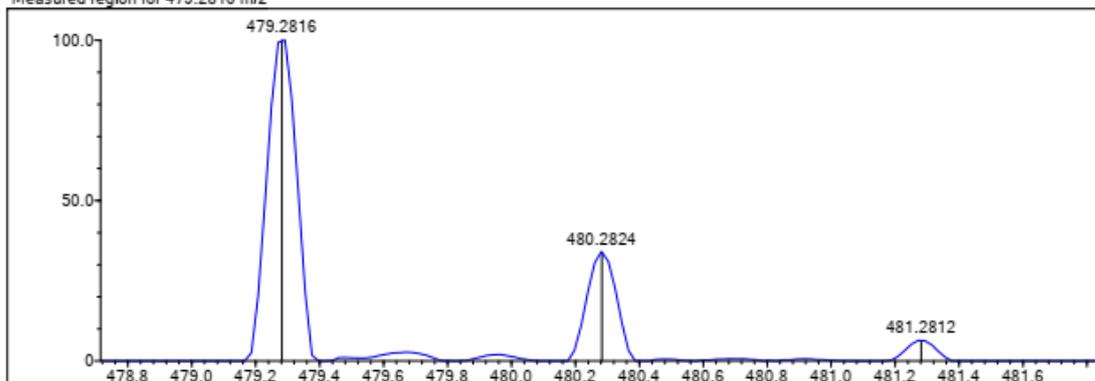
1 PDA Multi 1/254nm 4nm

PeakTable

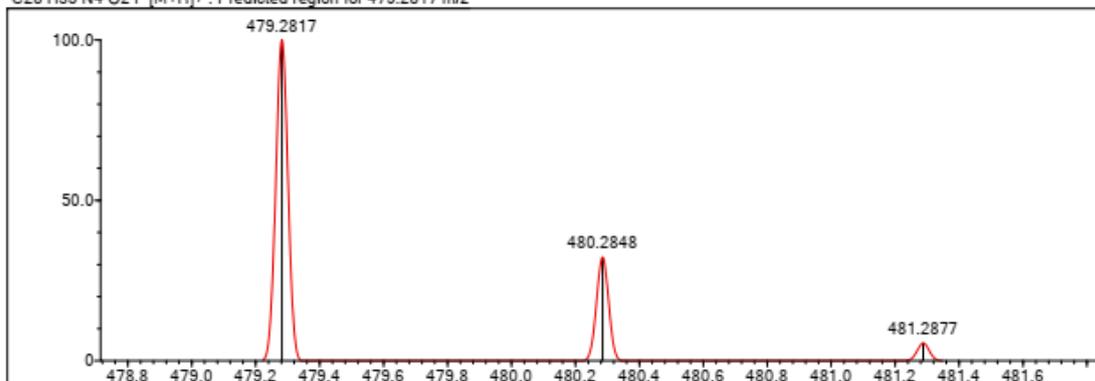
Peak#	Ret. Time	Area	Height	Area %	Height %
1	5.806	46214	3849	0.853	2.373
2	10.568	5369309	158344	99.147	97.627
Total		5415523	162193	100.000	100.000

HPLC analysis of 17f

Measured region for 479.2816 m/z

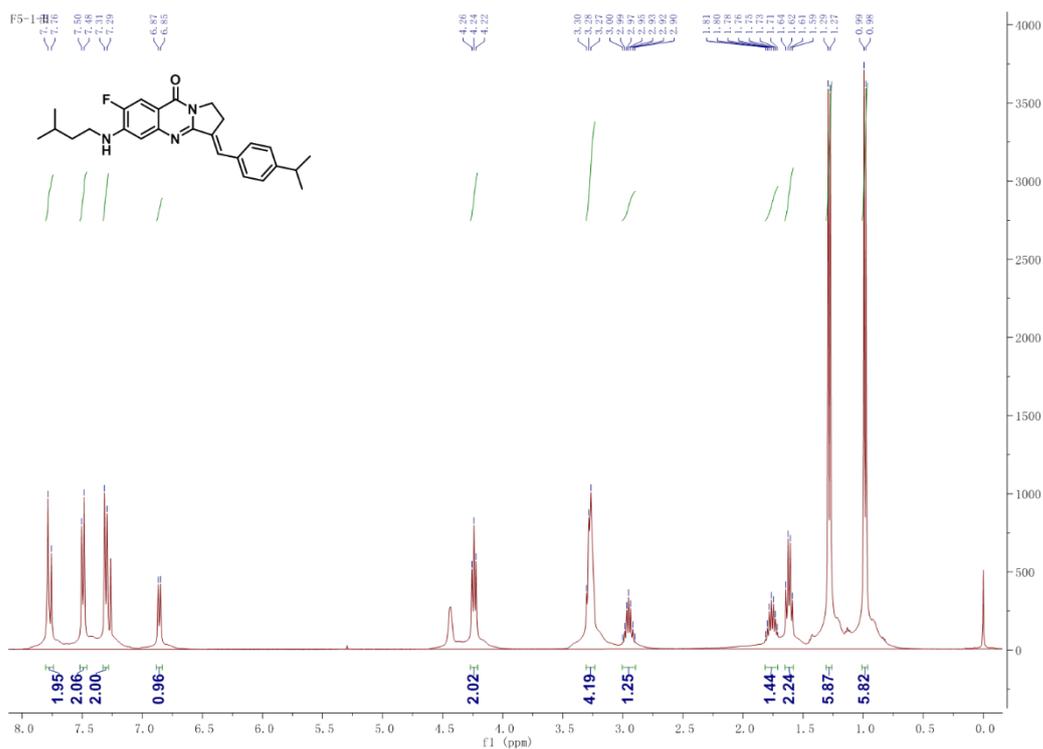


C28 H35 N4 O2 F [M+H]⁺ : Predicted region for 479.2817 m/z

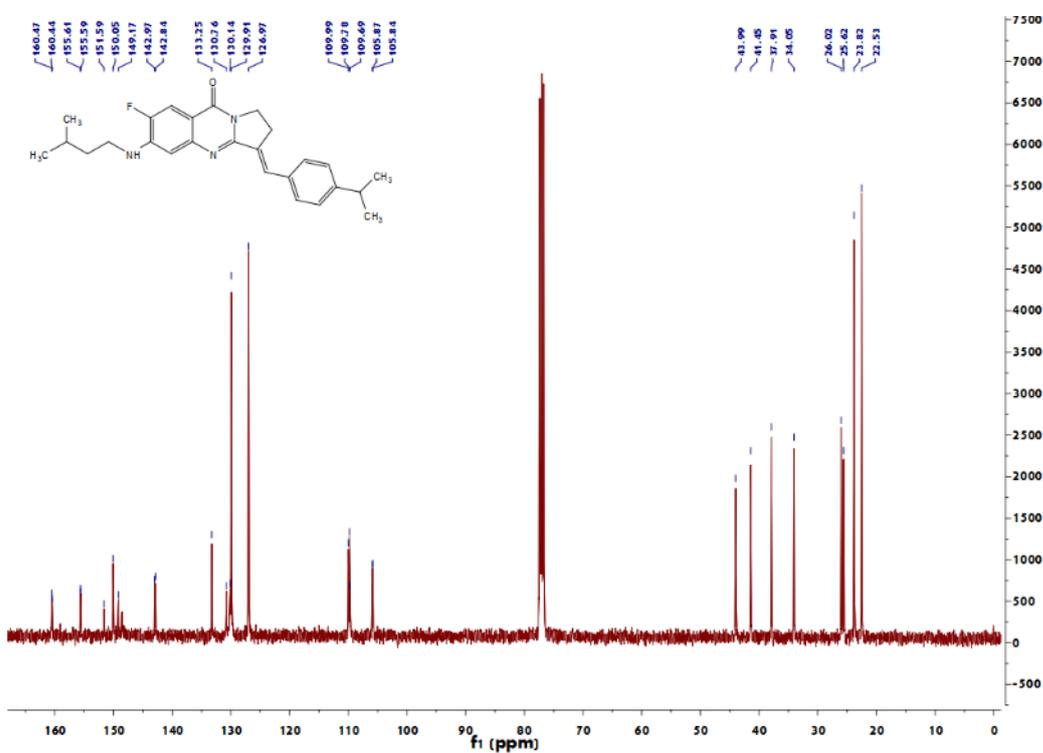


Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	80.43	C28 H35 N4 O2 F	[M+H] ⁺	479.2816	479.2817	-0.1	-0.21	80.43	13.0

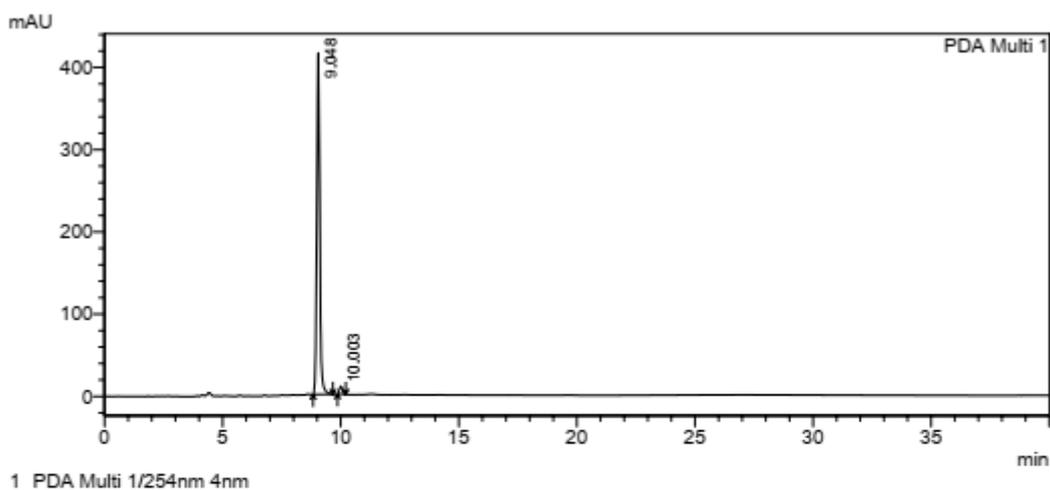
HRMS spectrum of 17f



^1H NMR spectrum of **18f**



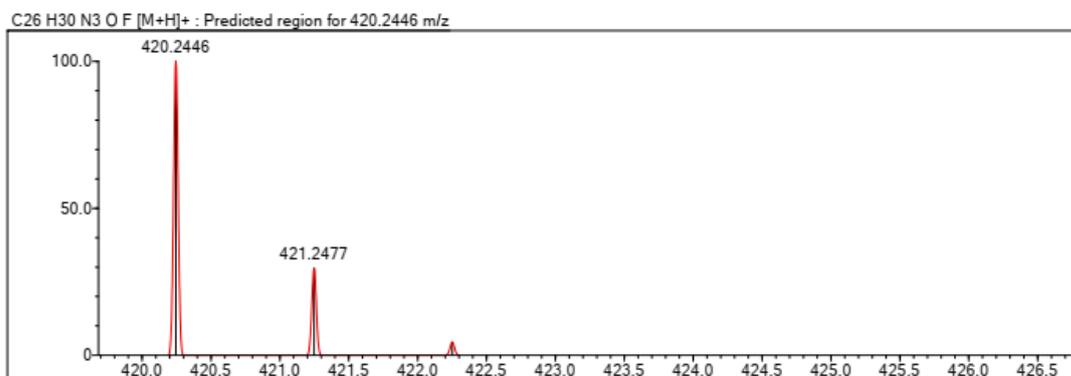
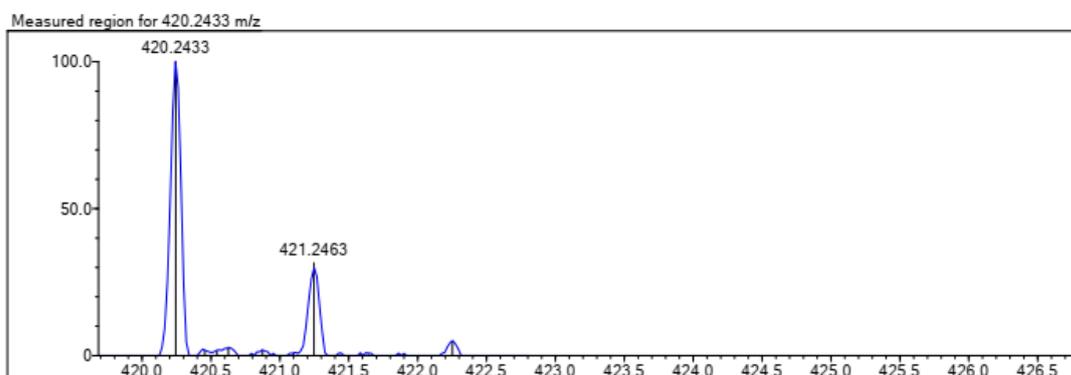
^{13}C NMR spectrum of **18f**



PeakTable

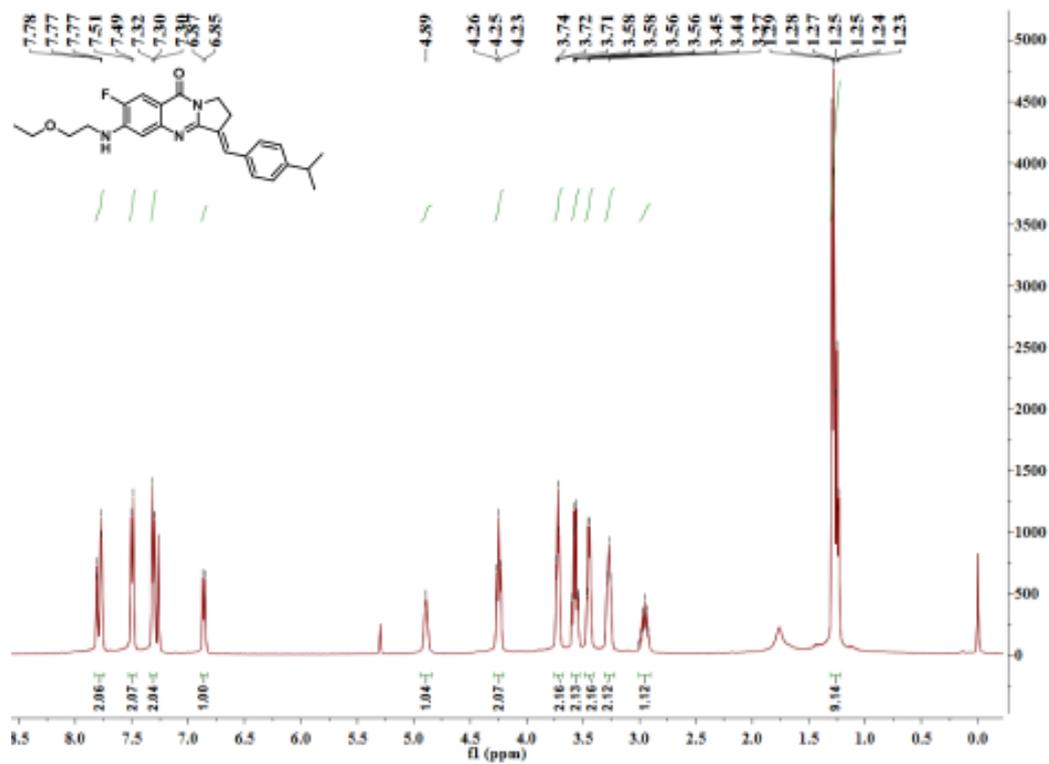
Peak#	Ret. Time	Area	Height	Area %	Height %
1	9.048	3632464	415181	97.734	97.750
2	10.003	84238	9556	2.266	2.250
Total		3716702	424737	100.000	100.000

HPLC analysis of 18f

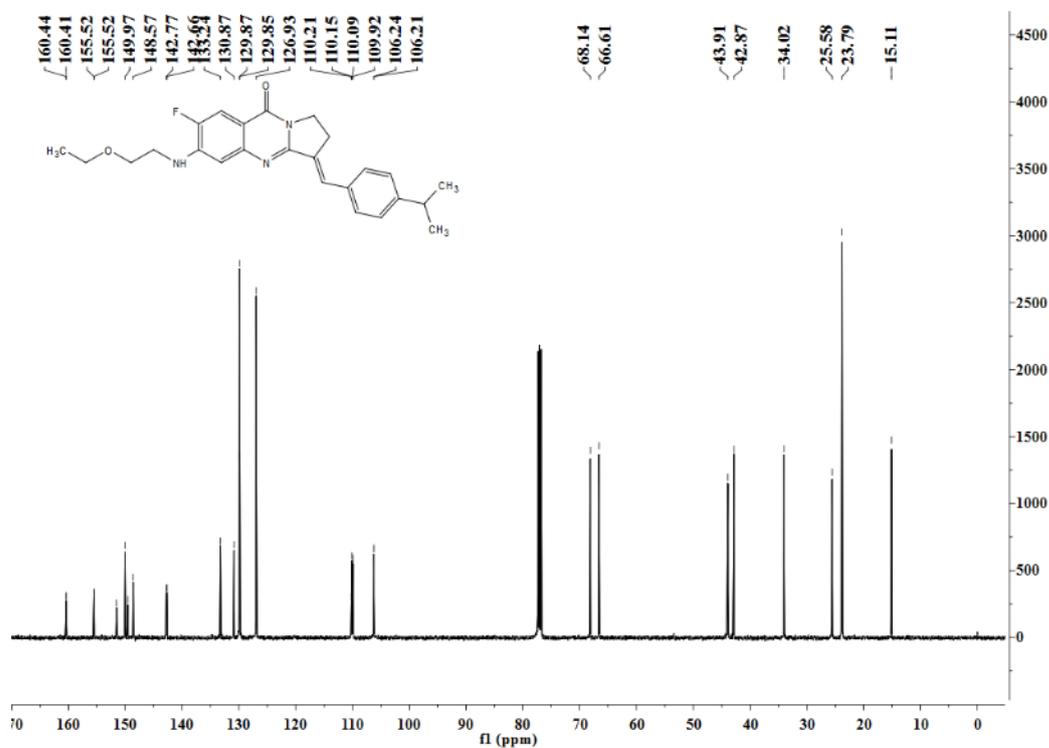


Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
2	80.13	C26 H30 N3 O F	[M+H] ⁺	420.2433	420.2446	-1.3	-3.09	84.55	13.0

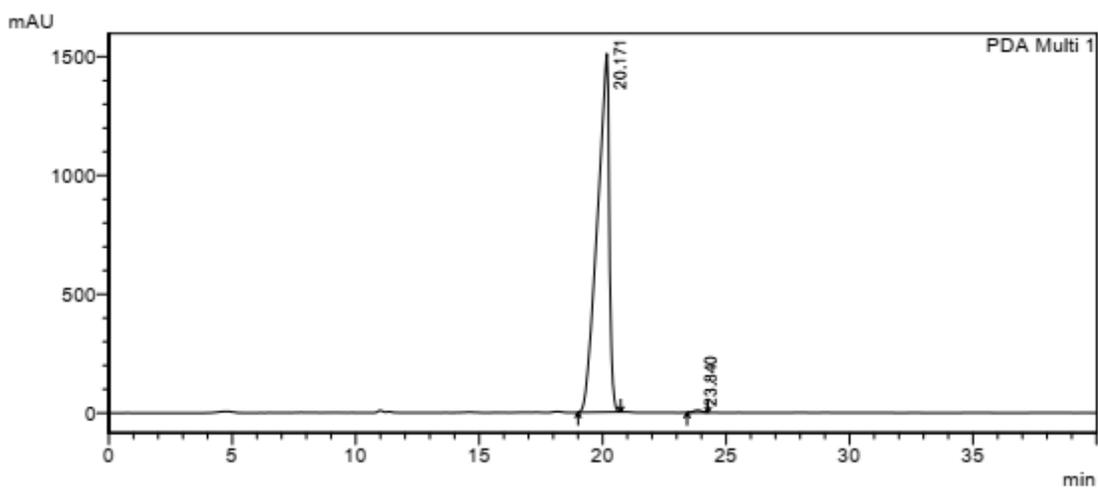
HRMS spectrum of 18f



¹H NMR spectrum of 19f



¹³C NMR spectrum of 19f

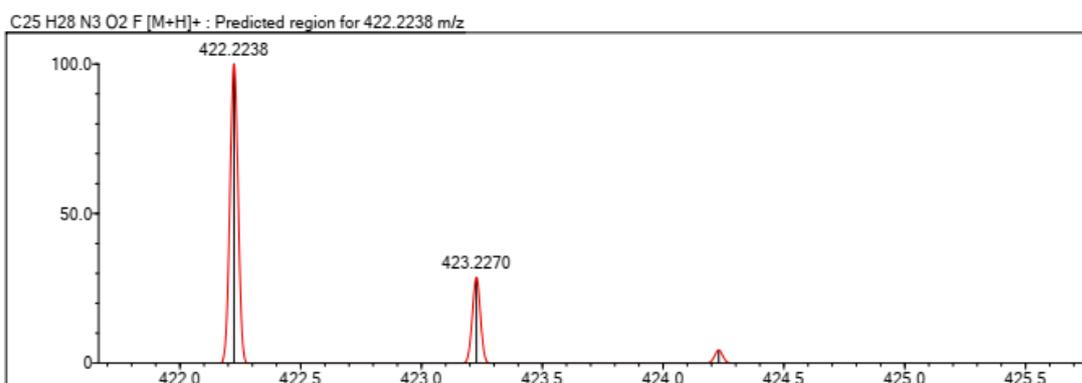
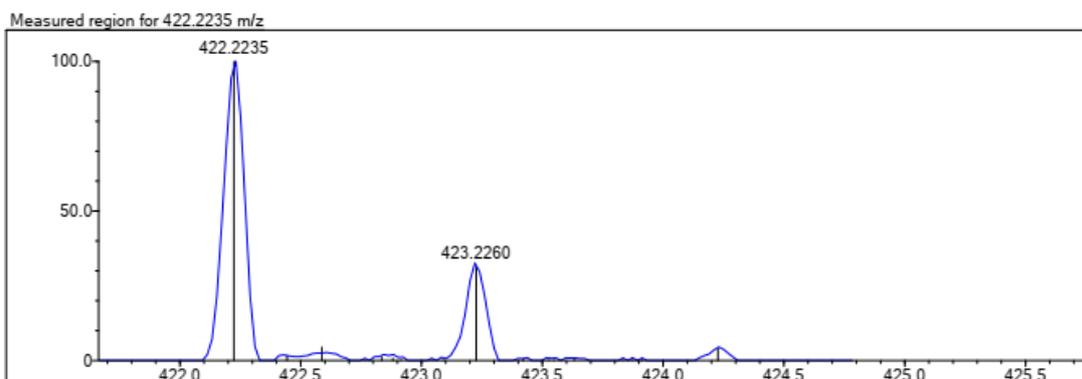


1 PDA Multi 1/254nm 4nm

PeakTable

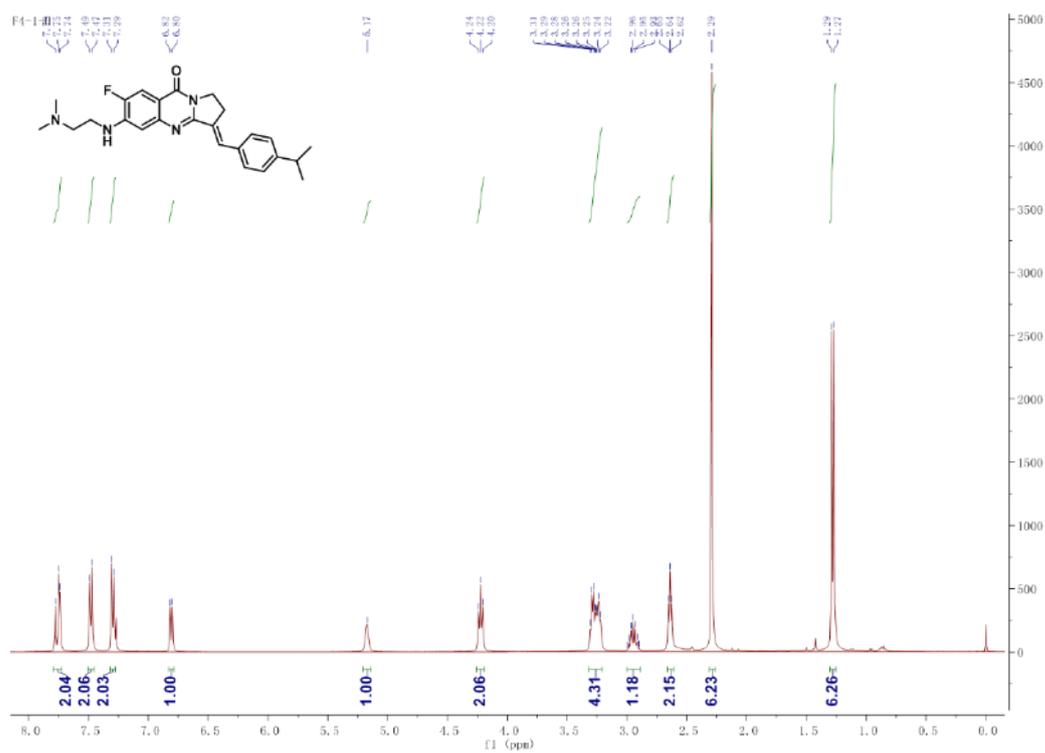
Peak#	Ret. Time	Area	Height	Area %	Height %
1	20.171	50485973	1509255	99.588	99.405
2	23.840	208809	9041	0.412	0.595
Total		50694782	1518296	100.000	100.000

HPLC analysis of 19f

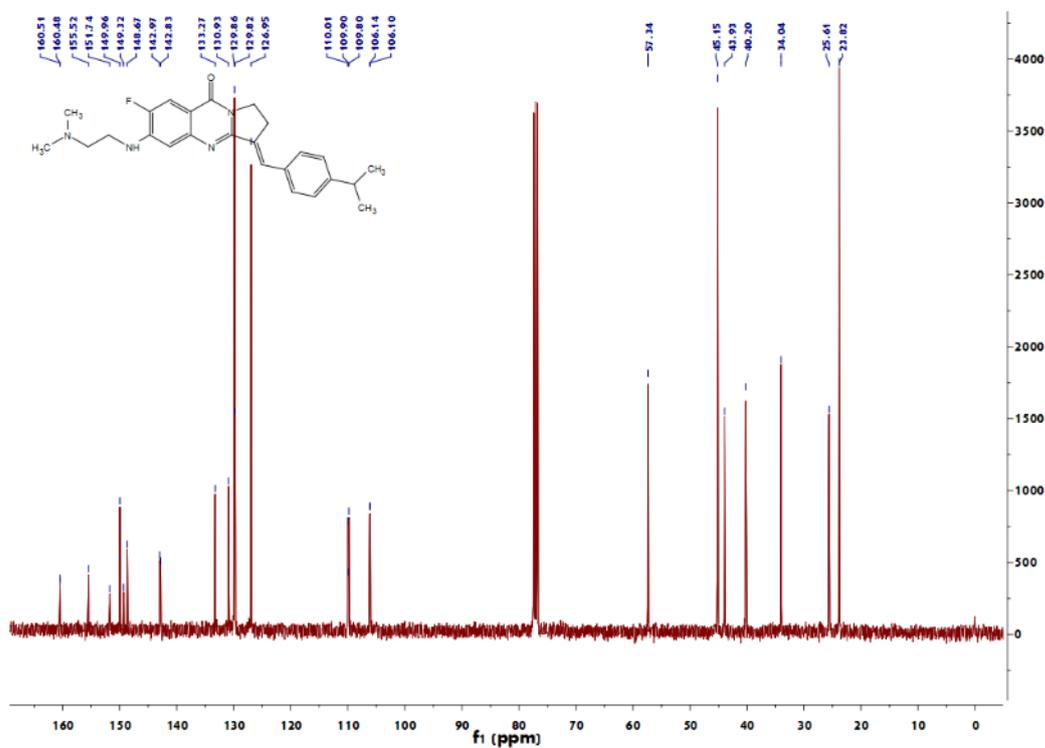


Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	77.64	C ₂₅ H ₂₈ N ₃ O ₂ F	[M+H] ⁺	422.2235	422.2238	-0.3	-0.71	77.64	13.0

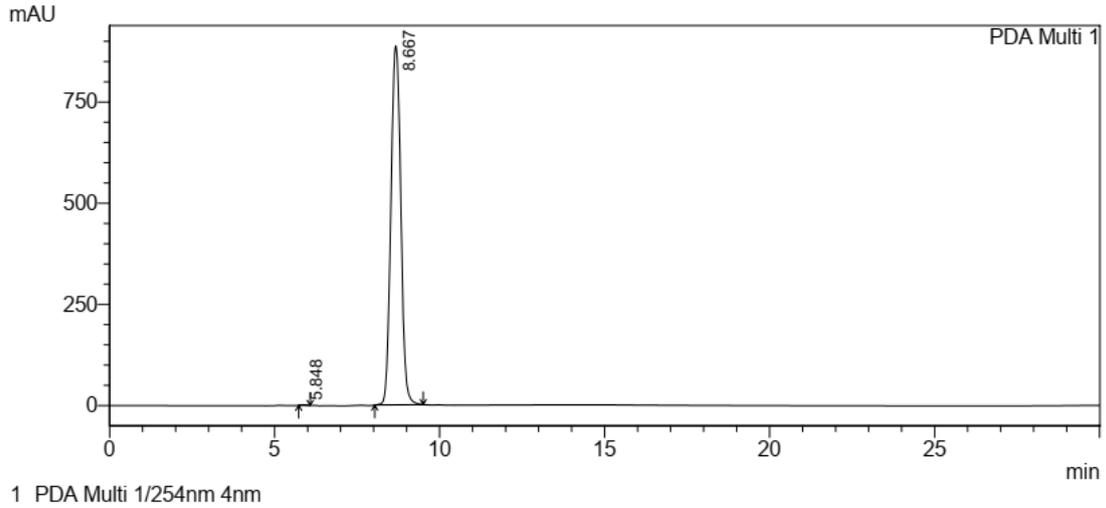
HRMS spectrum of 19f



¹H NMR spectrum of 20f



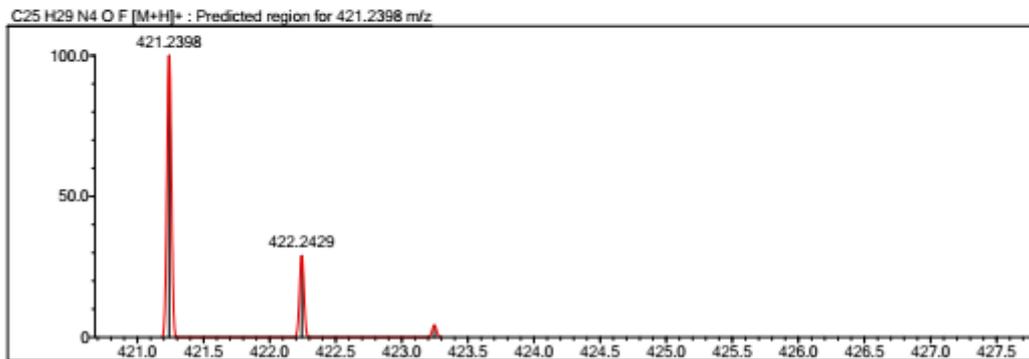
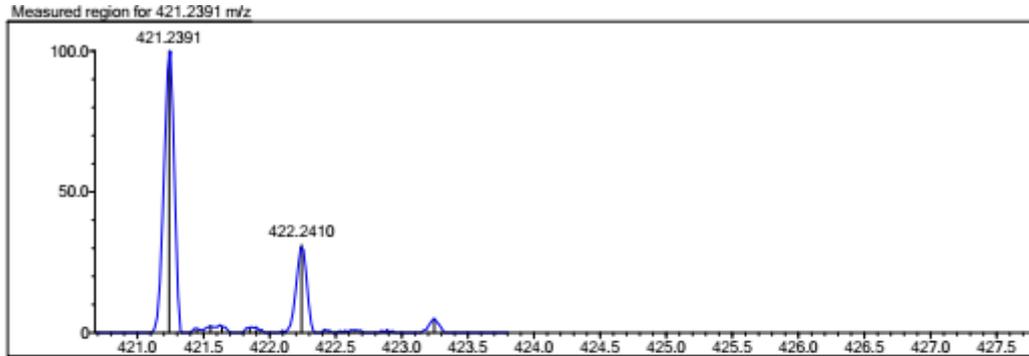
¹³C NMR spectrum of 20f



PeakTable

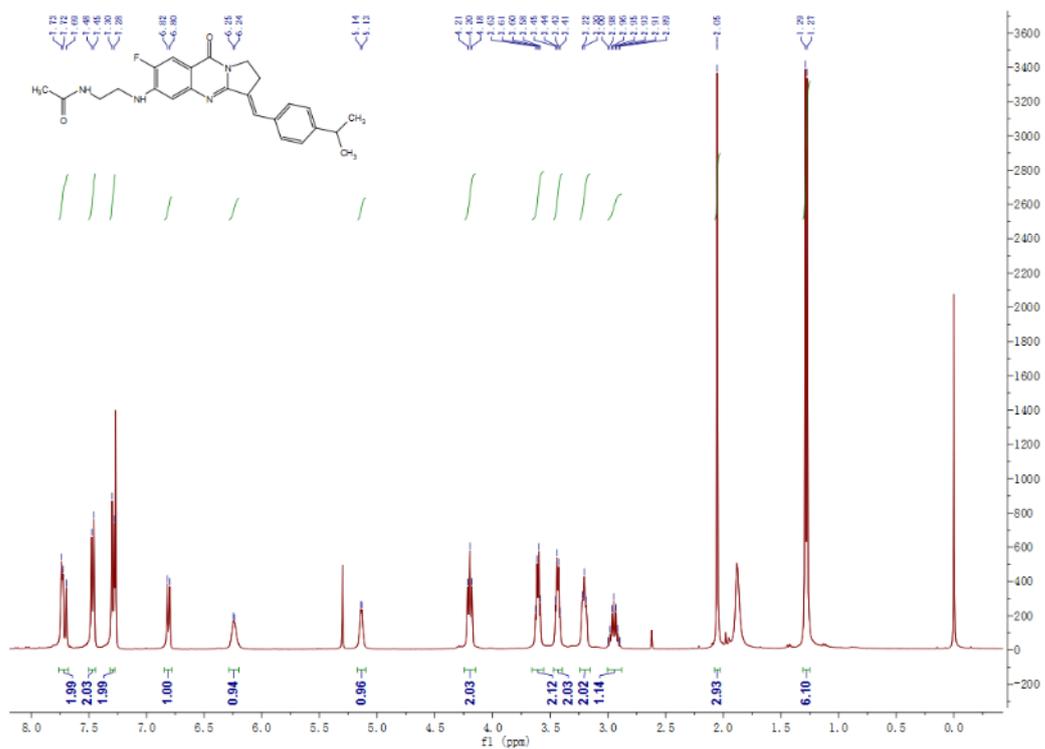
Peak#	Ret. Time	Area	Height	Area %	Height %
1	5.848	14943	1133	0.081	0.128
2	8.667	18521298	887622	99.919	99.872
Total		18536241	888755	100.000	100.000

HPLC analysis of 20f

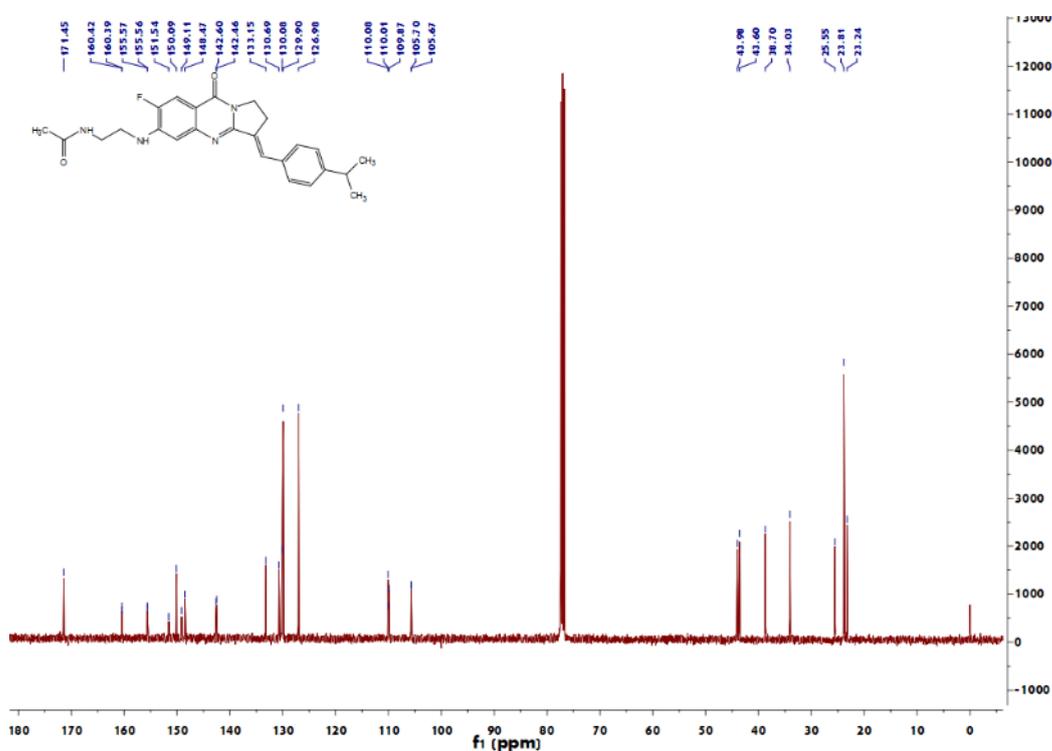


Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
2	77.37	C25 H29 N4 O F	[M+H] ⁺	421.2391	421.2398	-0.7	-1.66	78.67	13.0

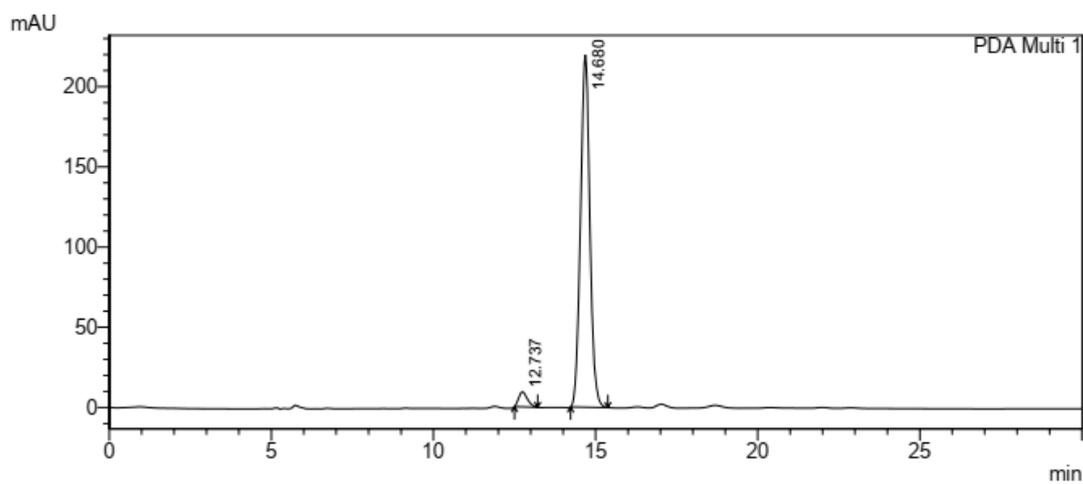
HRMS spectrum of 20f



¹H NMR spectrum of 21f



¹³C NMR spectrum of 21f

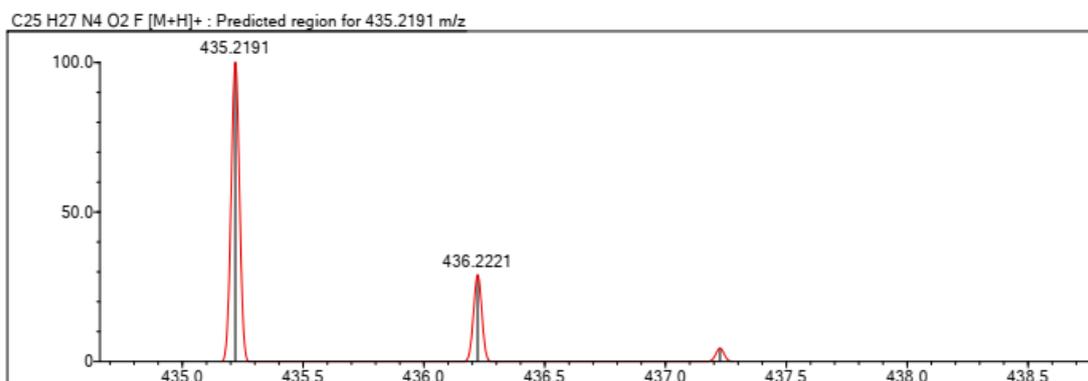
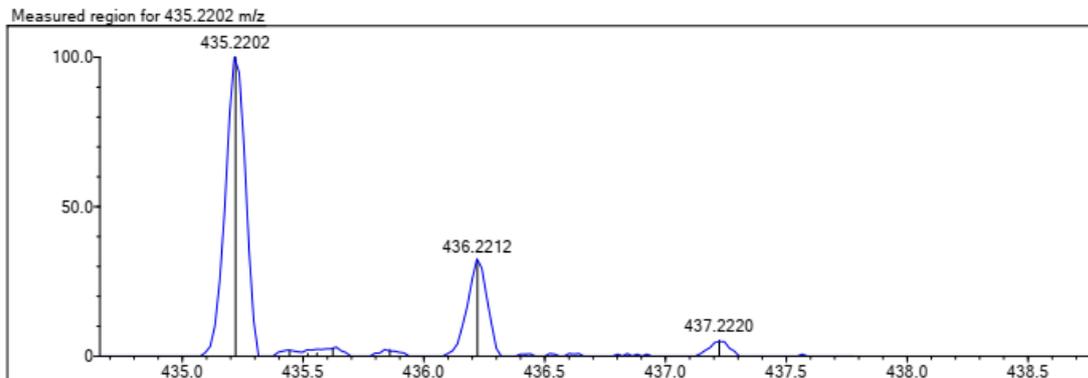


1 PDA Multi 1/254nm 4nm

PeakTable

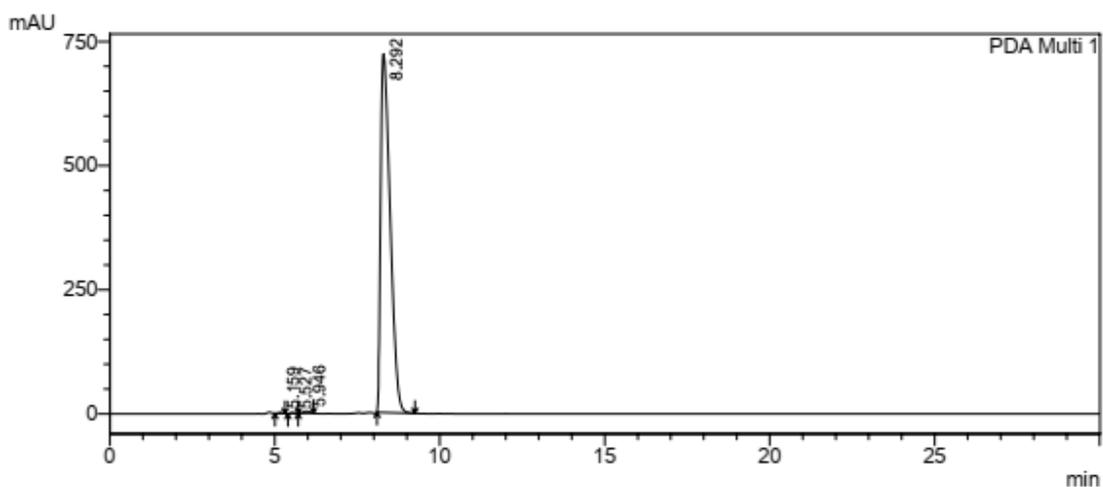
Peak#	Ret. Time	Area	Height	Area %	Height %
1	12.737	173839	9344	3.802	4.083
2	14.680	4398253	219514	96.198	95.917
Total		4572092	228858	100.000	100.000

HPLC analysis of 21f



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
4	75.15	C25 H27 N4 O2 F	[M+H] ⁺	435.2202	435.2191	1.1	2.53	78.14	14.0

HRMS spectrum of 21f



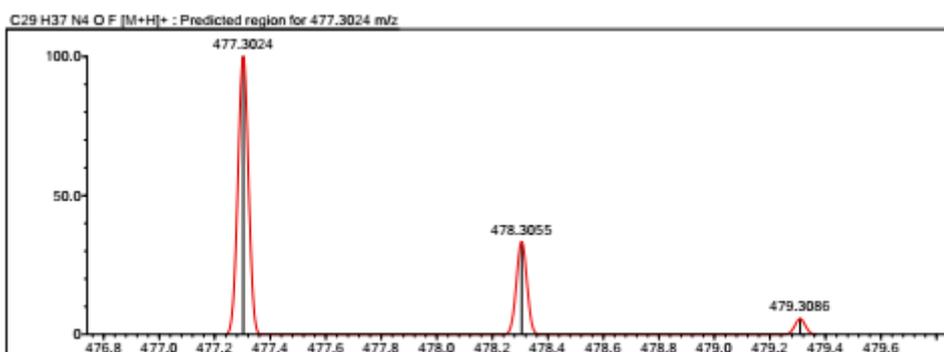
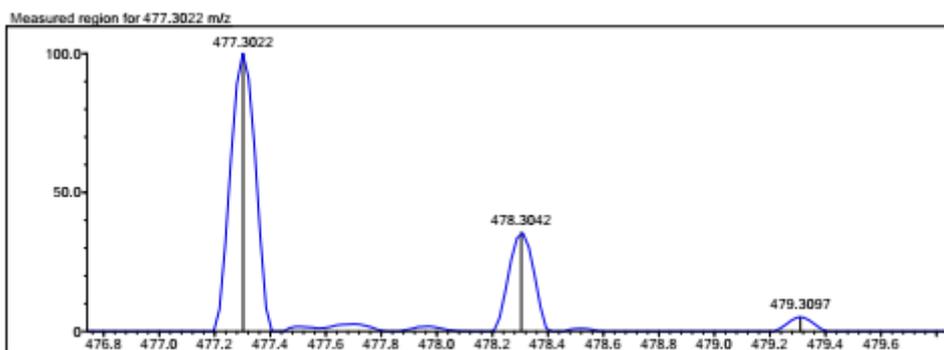
1 PDA Multi 1/254nm 4nm

PeakTable

PDA Chl 254nm 4nm

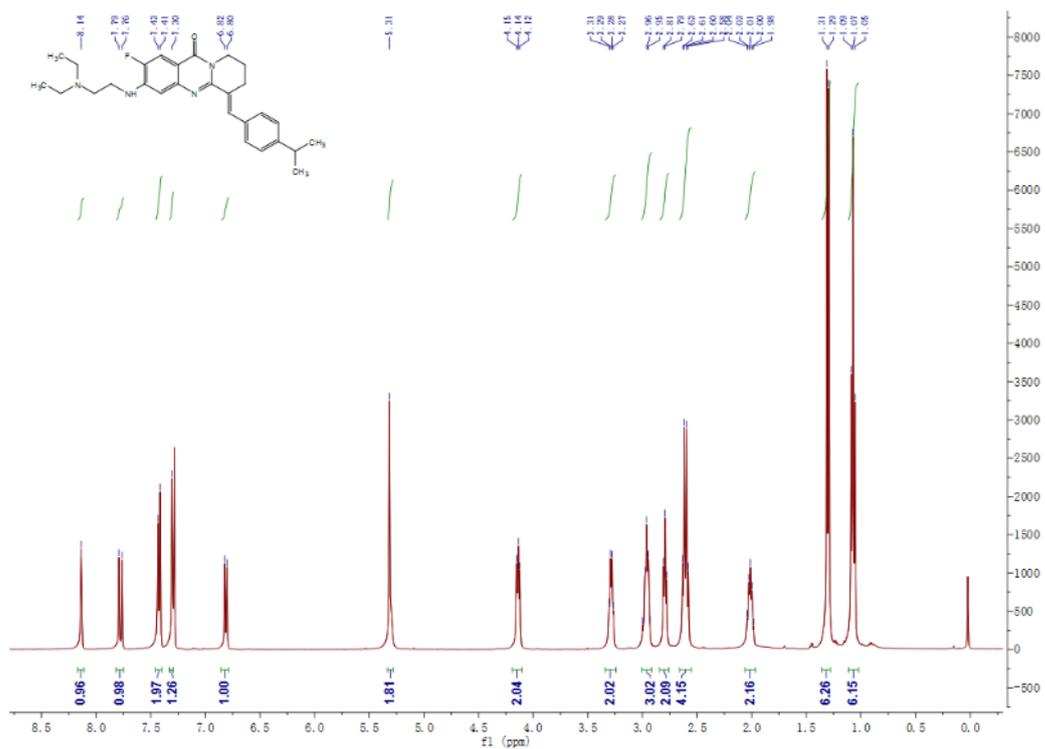
Peak#	Ret. Time	Area	Height	Area %	Height %
1	5.159	20229	2119	0.138	0.290
2	5.527	17529	1770	0.120	0.242
3	5.946	61007	4410	0.418	0.604
4	8.292	14512206	722210	99.324	98.864
Total		14610972	730509	100.000	100.000

HPLC analysis of 22f

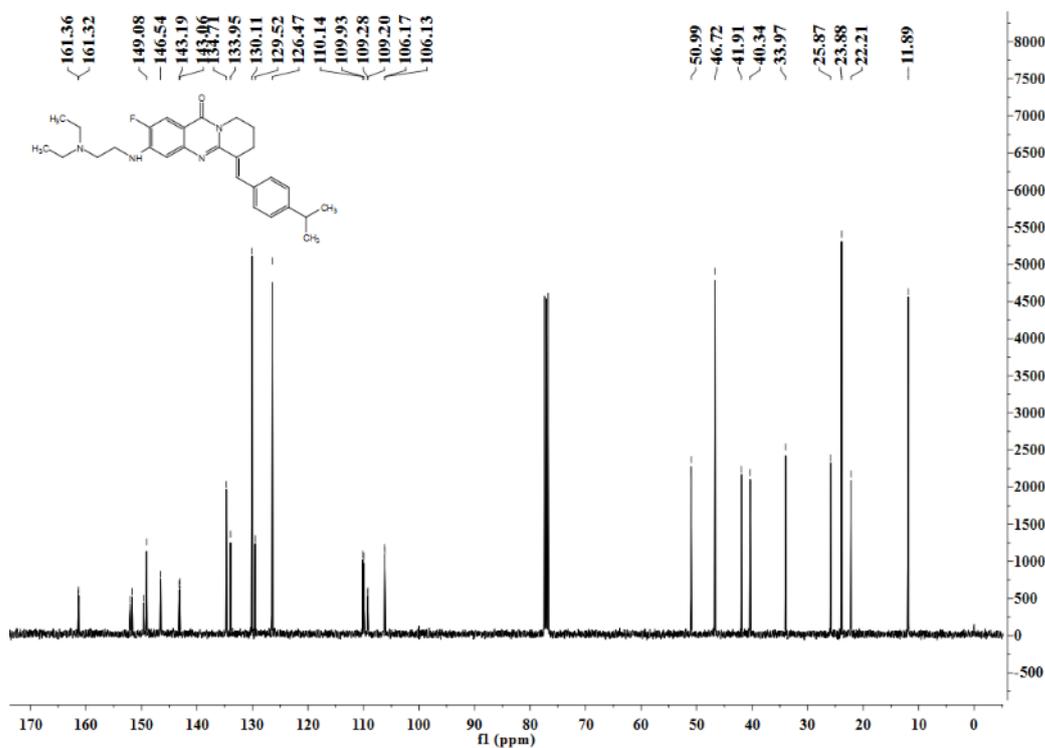


Rank	Score	Formula (M)	Ion	Mass. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Isot	DBE
1	89.54	C ₂₉ H ₃₇ N ₄ O F	[M+H] ⁺	477.3022	477.3024	-0.2	-0.42	89.54	13.0

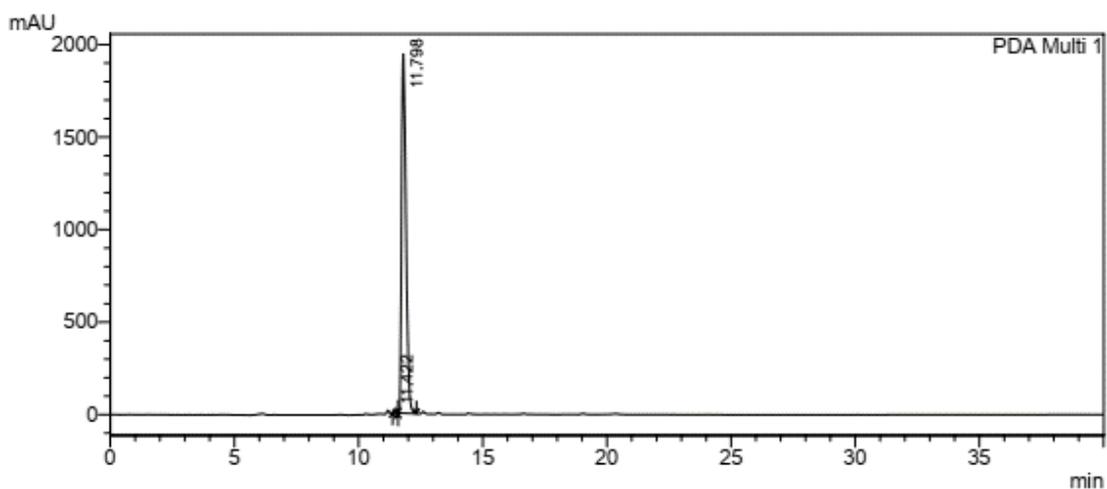
HRMS spectrum of 22f



¹H NMR spectrum of 23f



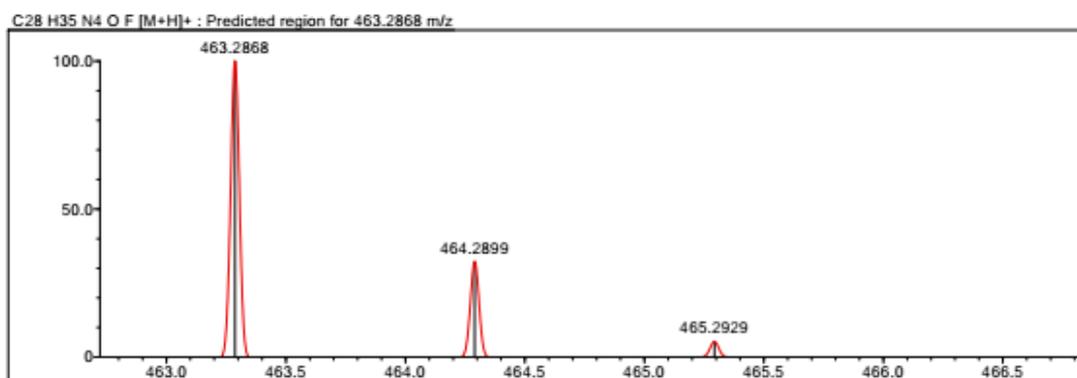
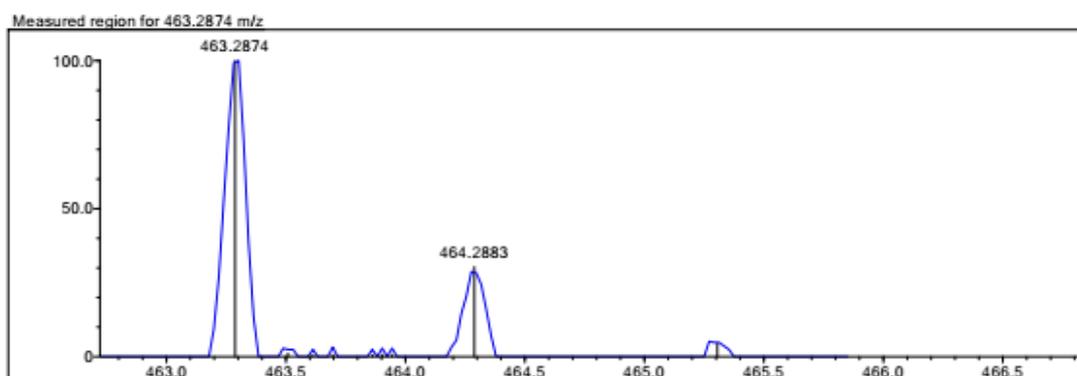
¹³C NMR spectrum of 23f



PeakTable

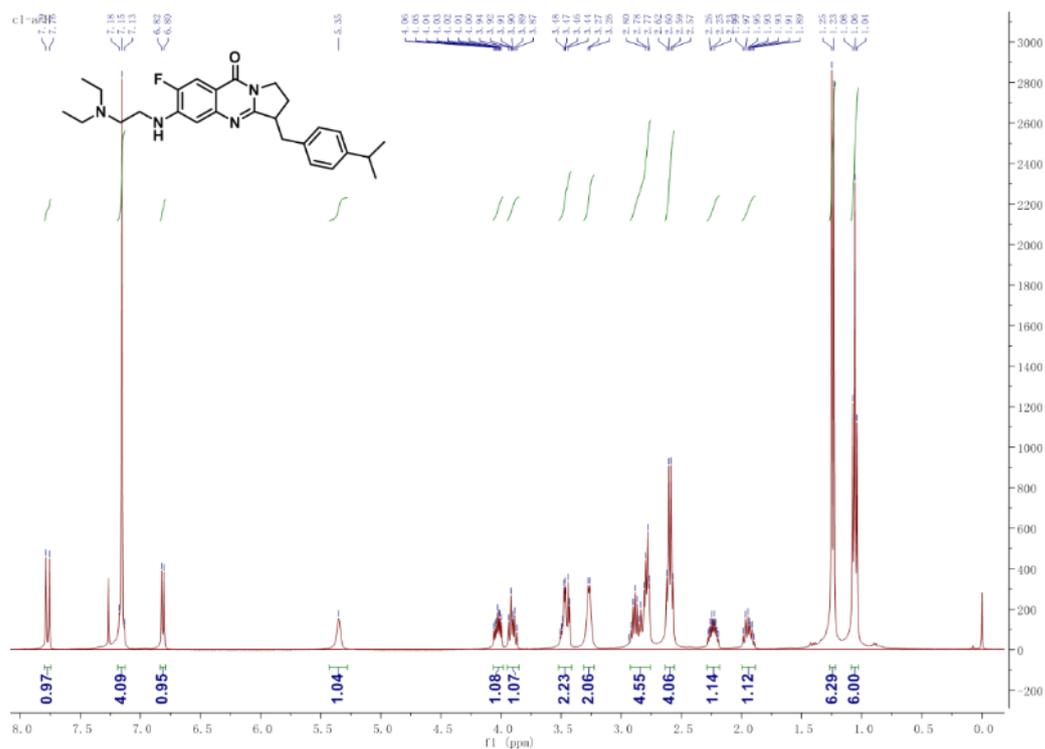
Peak#	Ret. Time	Area	Height	Area %	Height %
1	11.422	71557	14591	0.301	0.746
2	11.798	23718905	1941245	99.699	99.254
Total		23790462	1955836	100.000	100.000

HPLC analysis of 23f

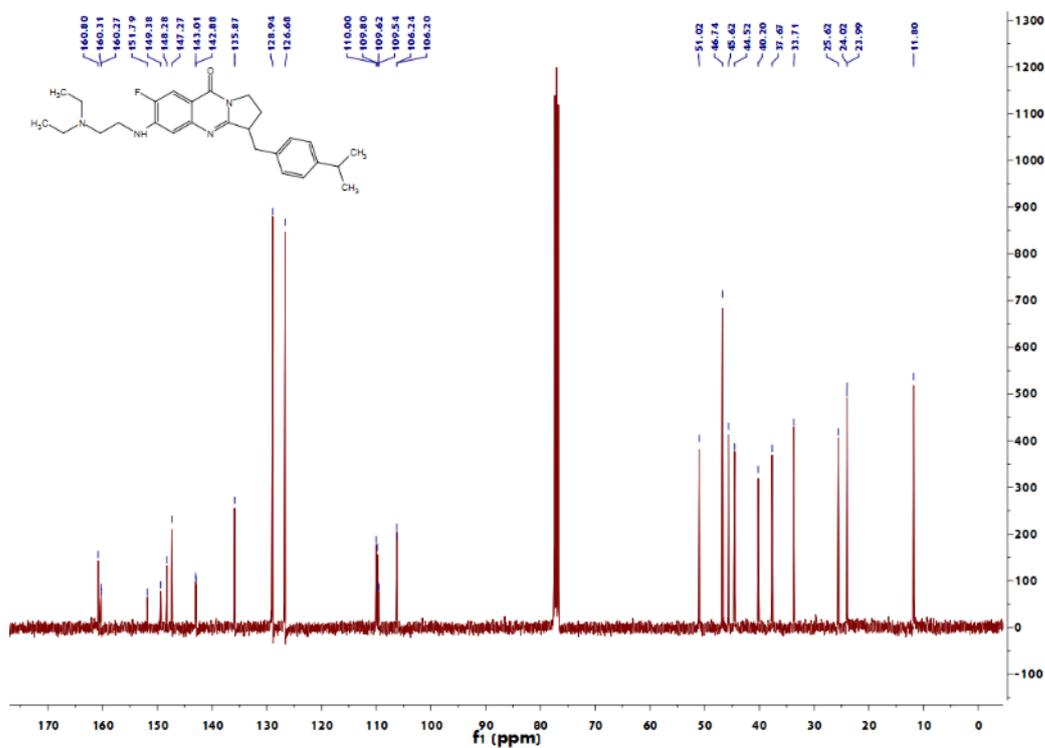


Rank	Score	Formula (M)	Ion	Mass. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
2	91.08	C28 H35 N4 O F	[M+H] ⁺	463.2874	463.2868	0.6	1.30	91.76	13.0

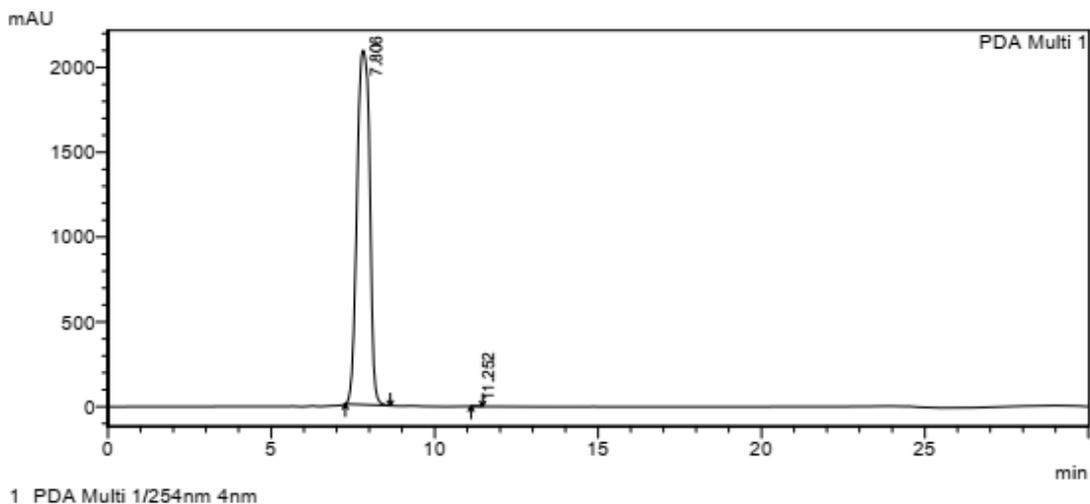
HRMS spectrum of 23f



¹H NMR spectrum of **1g**



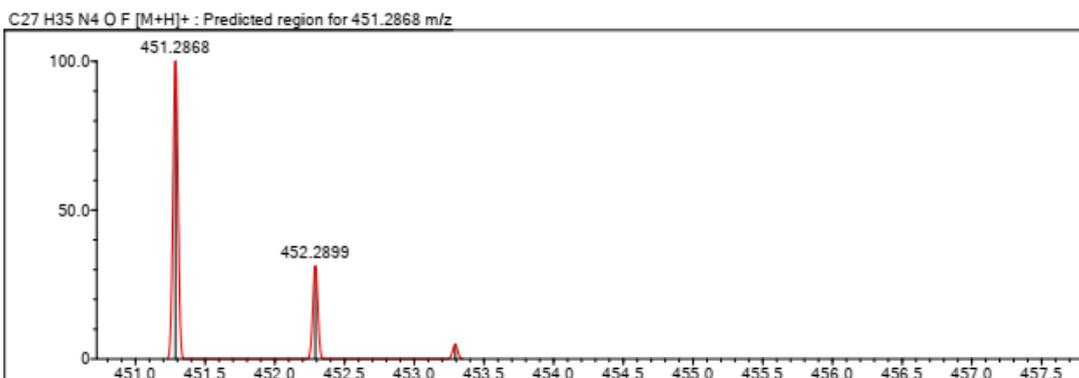
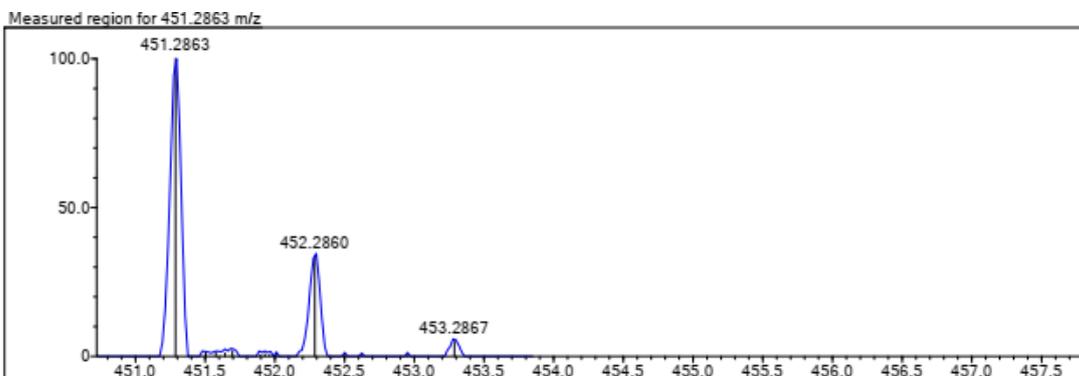
¹³C NMR spectrum of **1g**



PeakTable

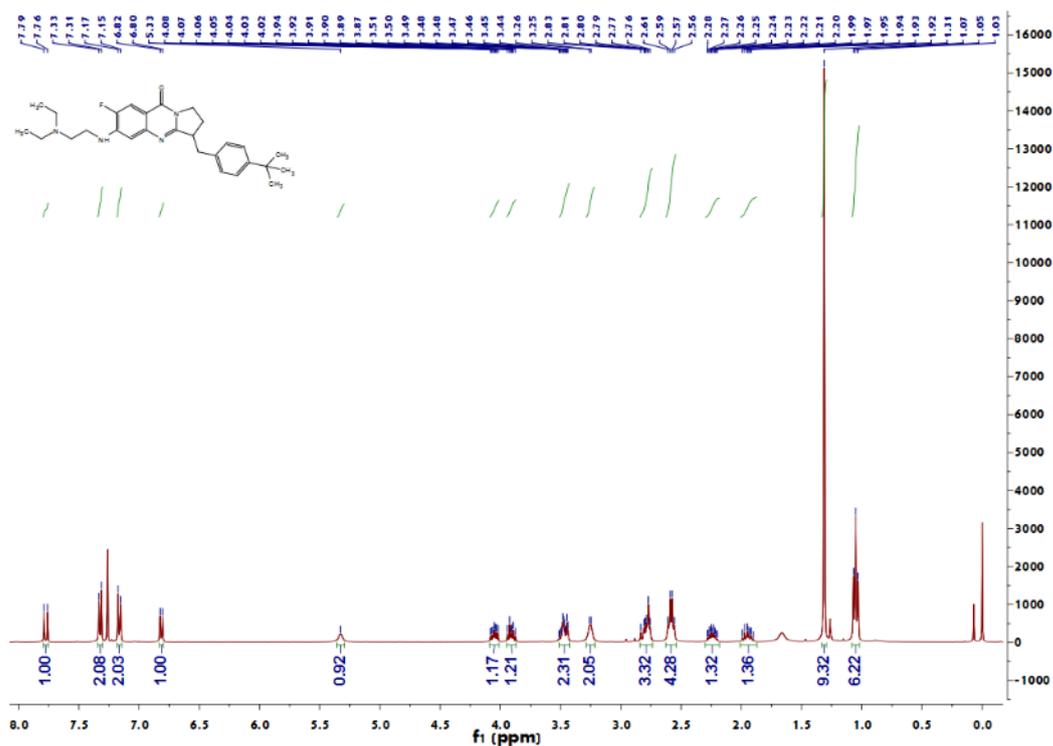
Peak#	Ret. Time	Area	Height	Area %	Height %
1	7.806	56524988	2089097	99.960	99.918
2	11.252	22425	1716	0.040	0.082
Total		56547414	2090813	100.000	100.000

HPLC analysis of **1g**

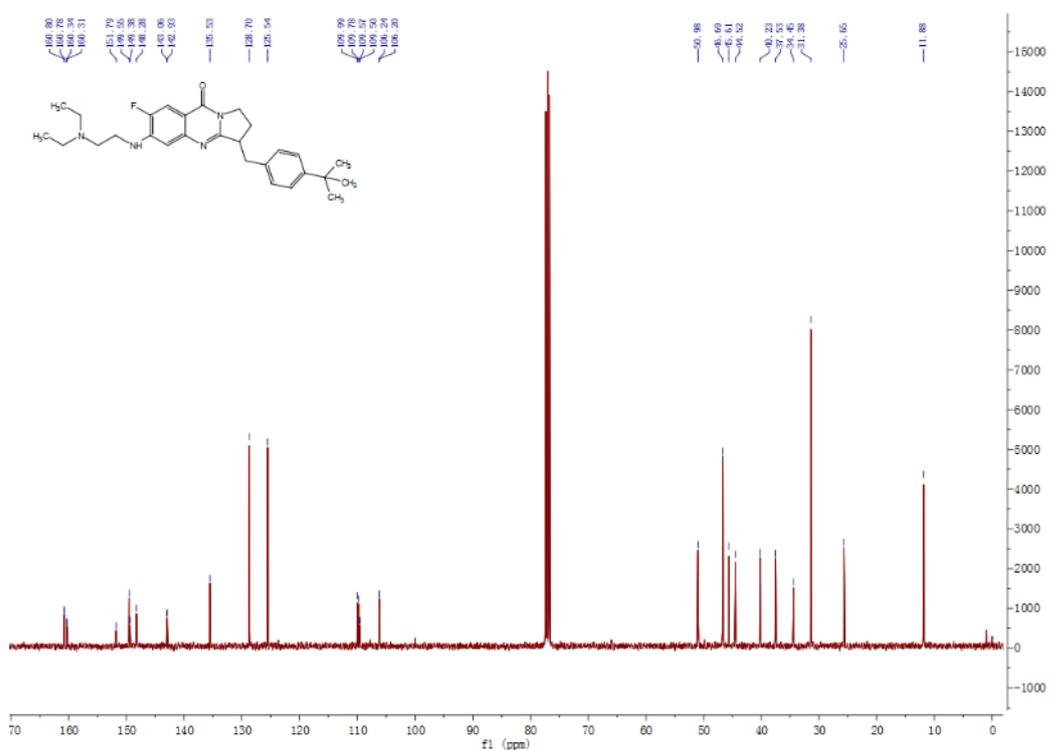


Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Isot	DBE
2	86.59	C27 H35 N4 O F	[M+H] ⁺	451.2863	451.2868	-0.5	-1.11	86.83	12.0

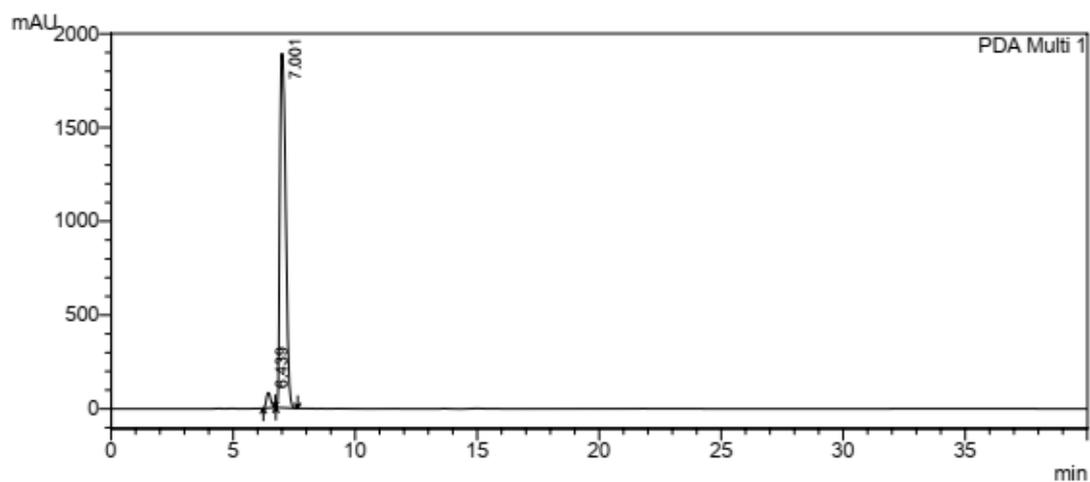
HRMS spectrum of **1g**



¹H NMR spectrum of **2g**



¹³C NMR spectrum of **2g**

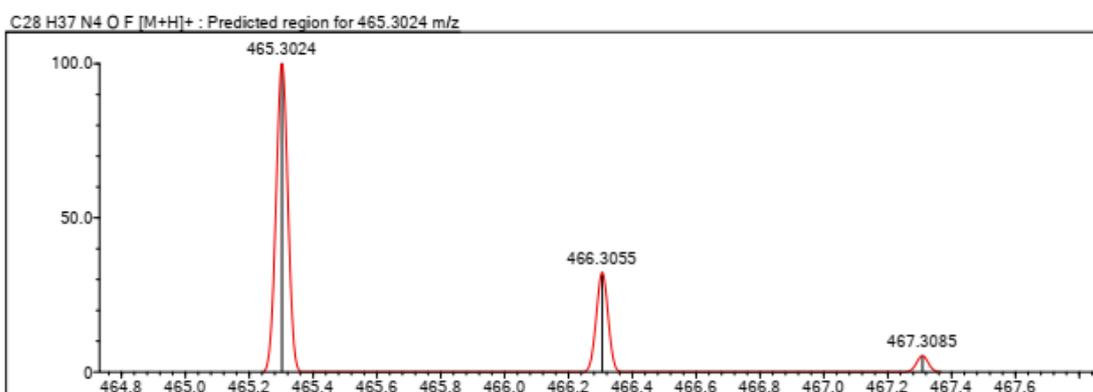
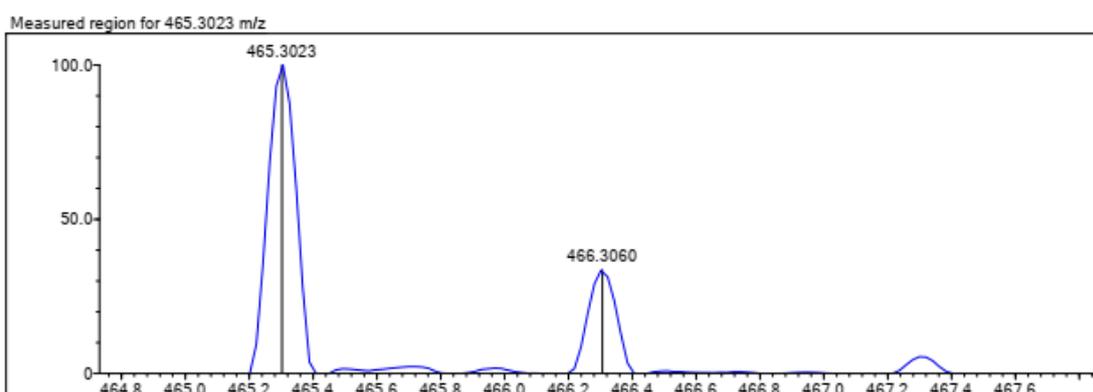


1 PDA Multi 1/254nm 4nm

PeakTable

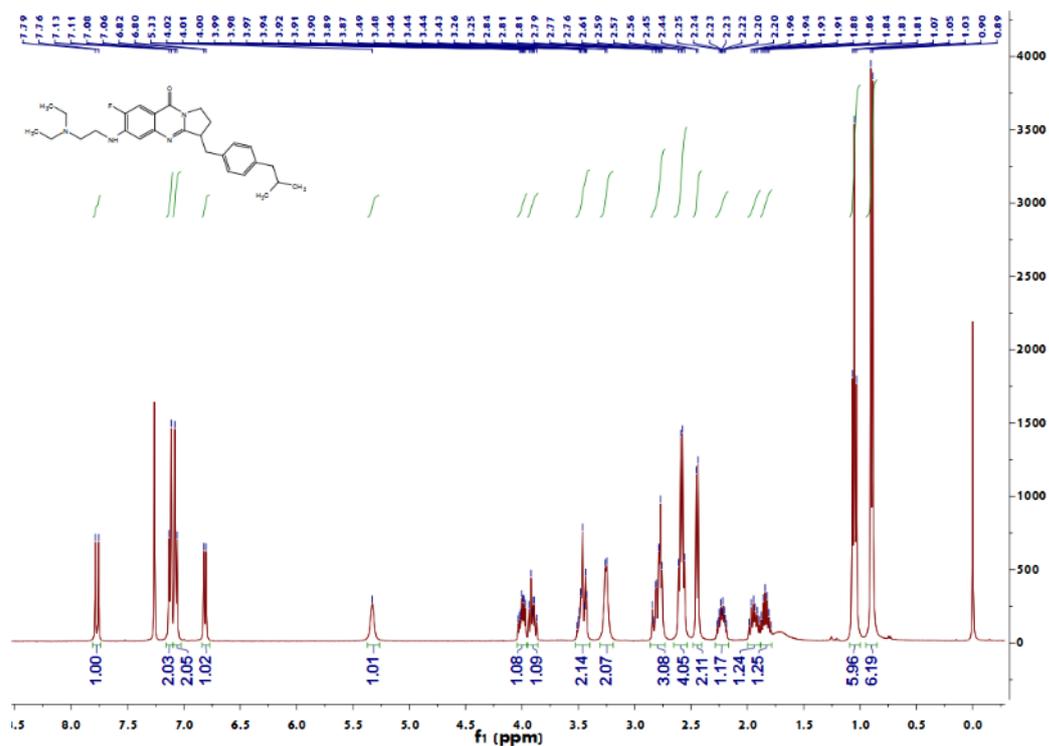
Peak#	Ret. Time	Area	Height	Area %	Height %
1	6.439	1026786	80867	3.211	4.104
2	7.001	30954131	1889489	96.789	95.896
Total		31980917	1970357	100.000	100.000

HPLC analysis of 2g

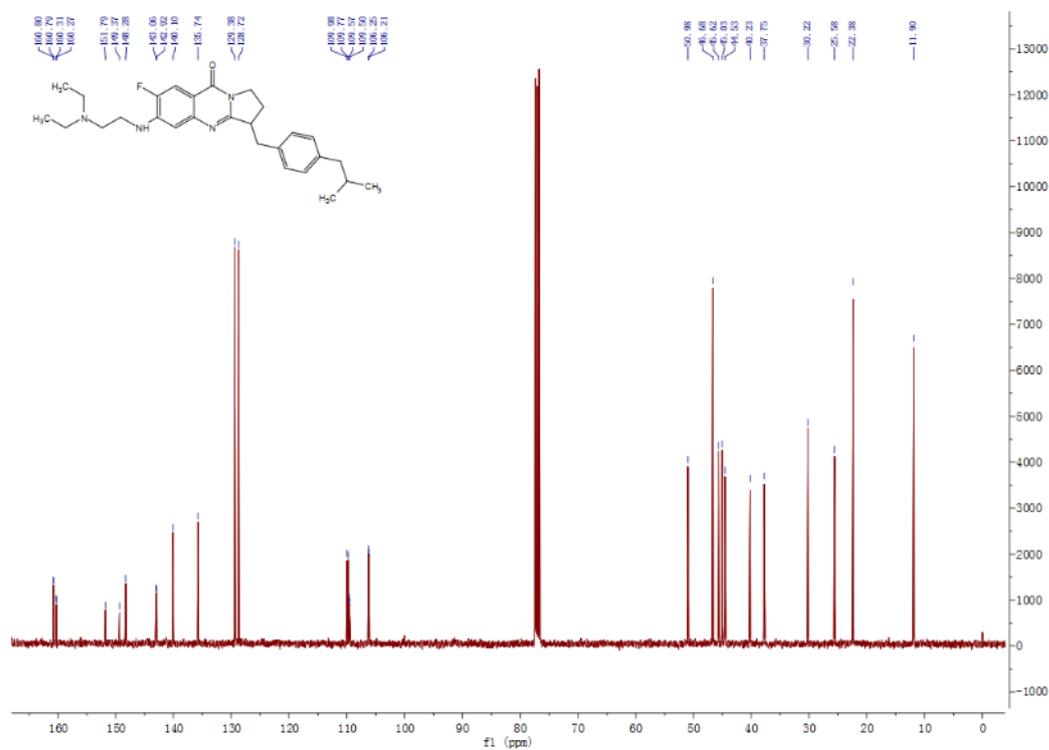


Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	88.82	C28 H37 N4 O F	[M+H] ⁺	465.3023	465.3024	-0.1	-0.21	88.82	12.0

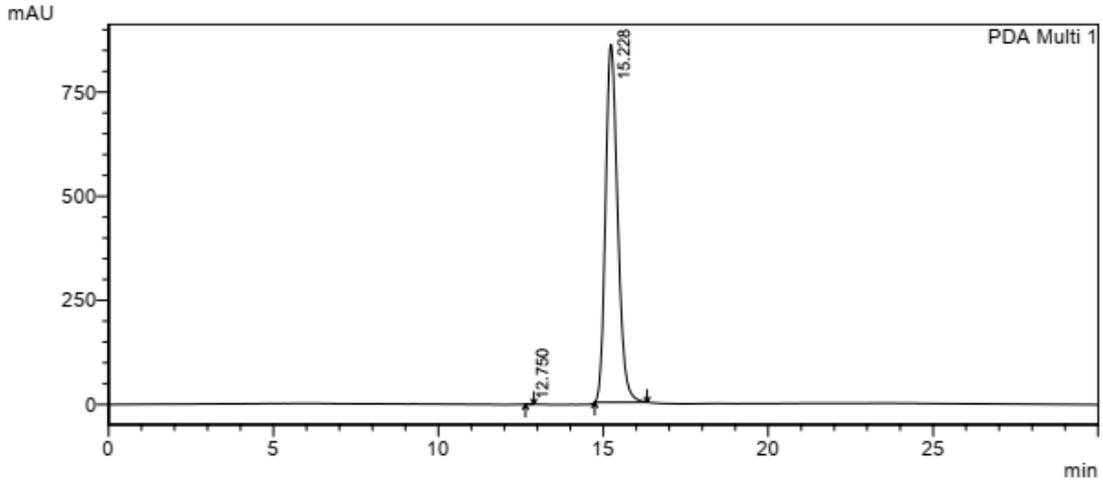
HRMS spectrum of 2g



¹H NMR spectrum of 3g



¹³C NMR spectrum of 3g

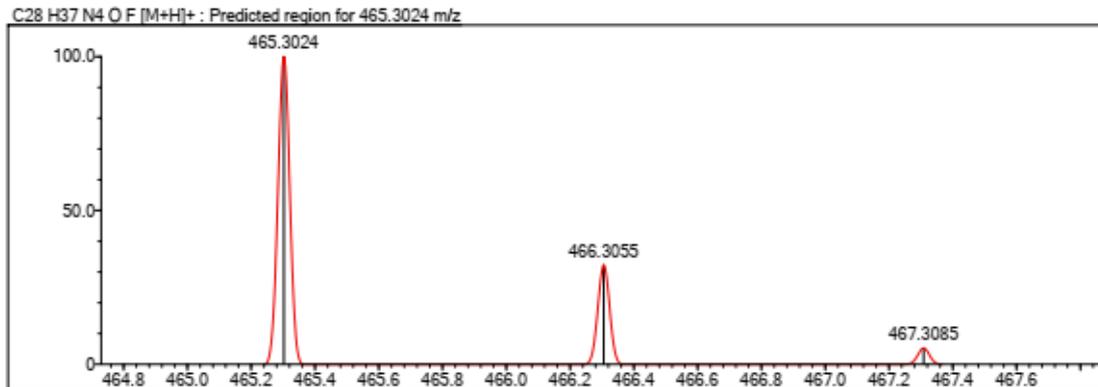
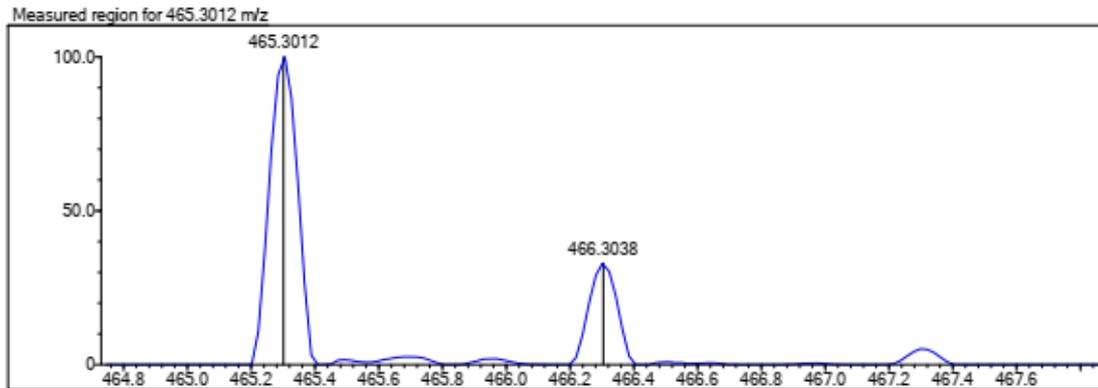


1 PDA Multi 1/254nm 4nm

PeakTable

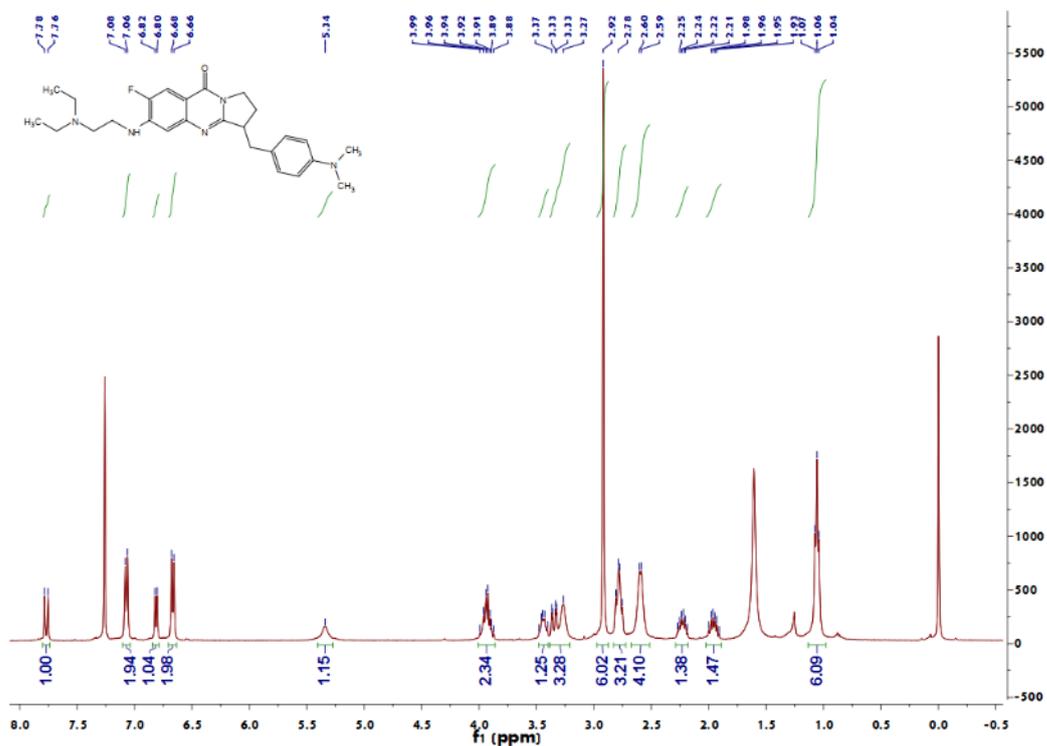
Peak#	Ret. Time	Area	Height	Area %	Height %
1	12.750	4405	454	0.020	0.053
2	15.228	22013997	859425	99.980	99.947
Total		22018402	859879	100.000	100.000

HPLC analysis of 3g

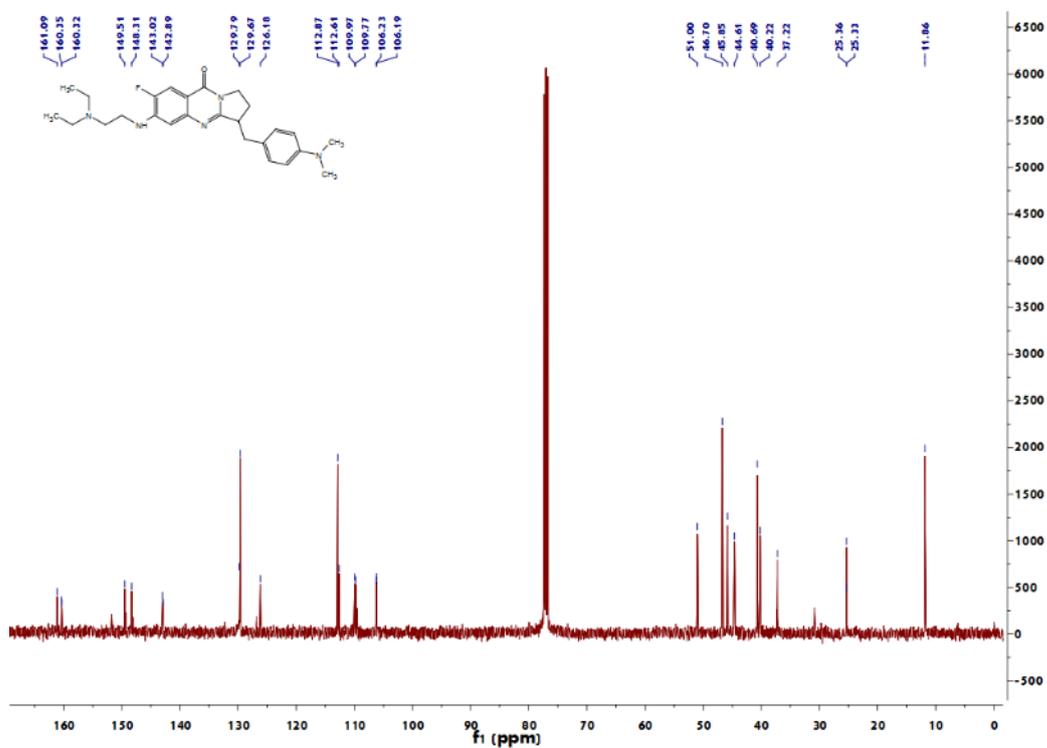


Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
5	83.67	C28 H37 N4 O F	[M+H] ⁺	465.3012	465.3024	-1.2	-2.58	87.12	12.0

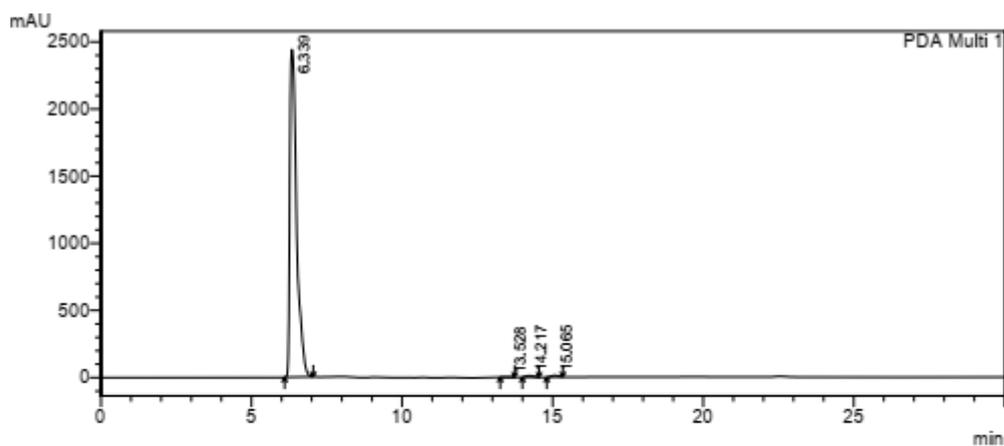
HRMS spectrum of 3g



¹H NMR spectrum of **4g**



¹³C NMR spectrum of **4g**

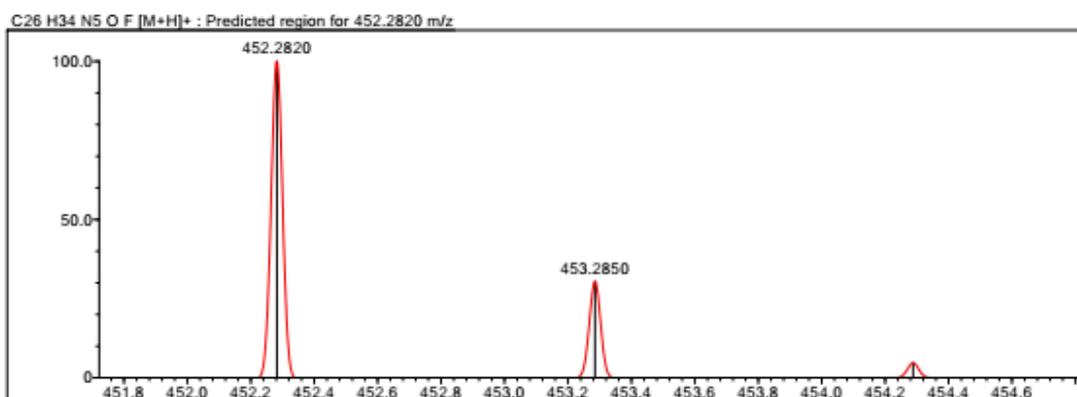
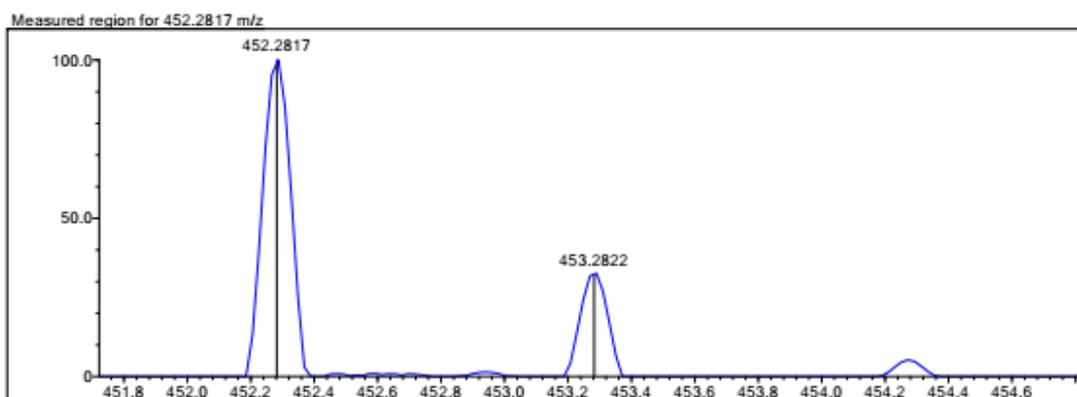


1 PDA Multi 1/254nm 4nm

PeakTable

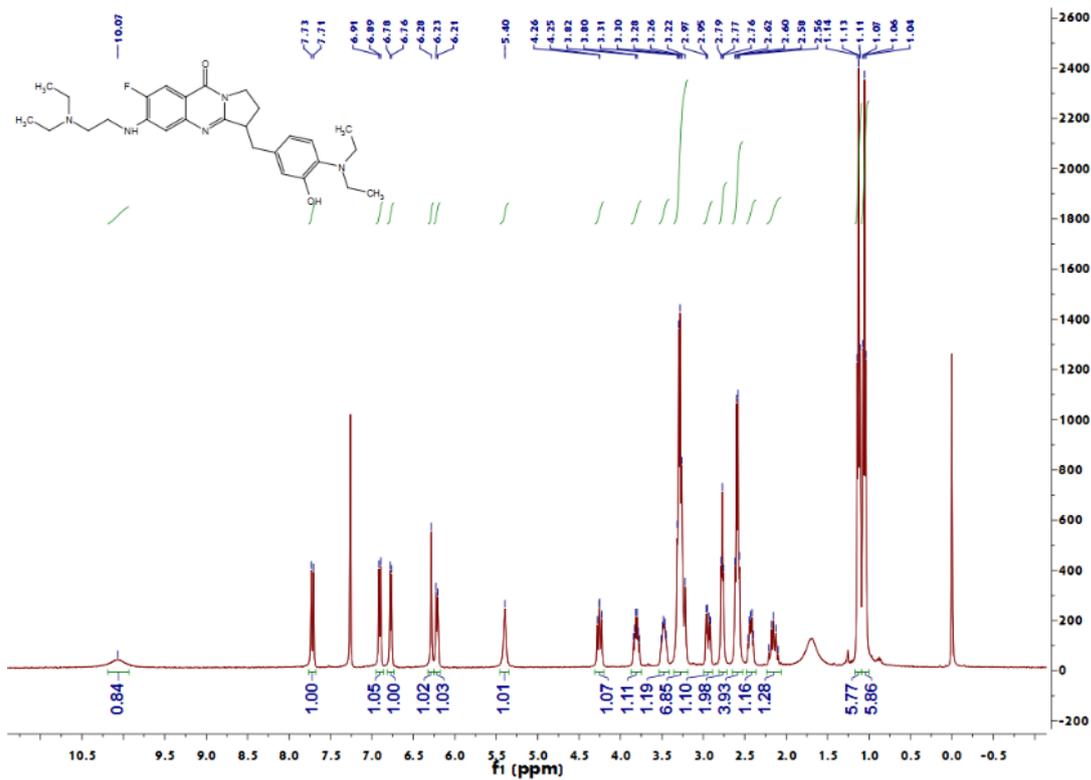
Peak#	Ret. Time	Area	Height	Area %	Height %
1	6.339	37915606	2440040	98.896	98.953
2	13.528	15053	818	0.039	0.033
3	14.217	204024	12036	0.532	0.488
4	15.065	204151	12968	0.532	0.526
Total		38338834	2465862	100.000	100.000

HPLC analysis of 4g

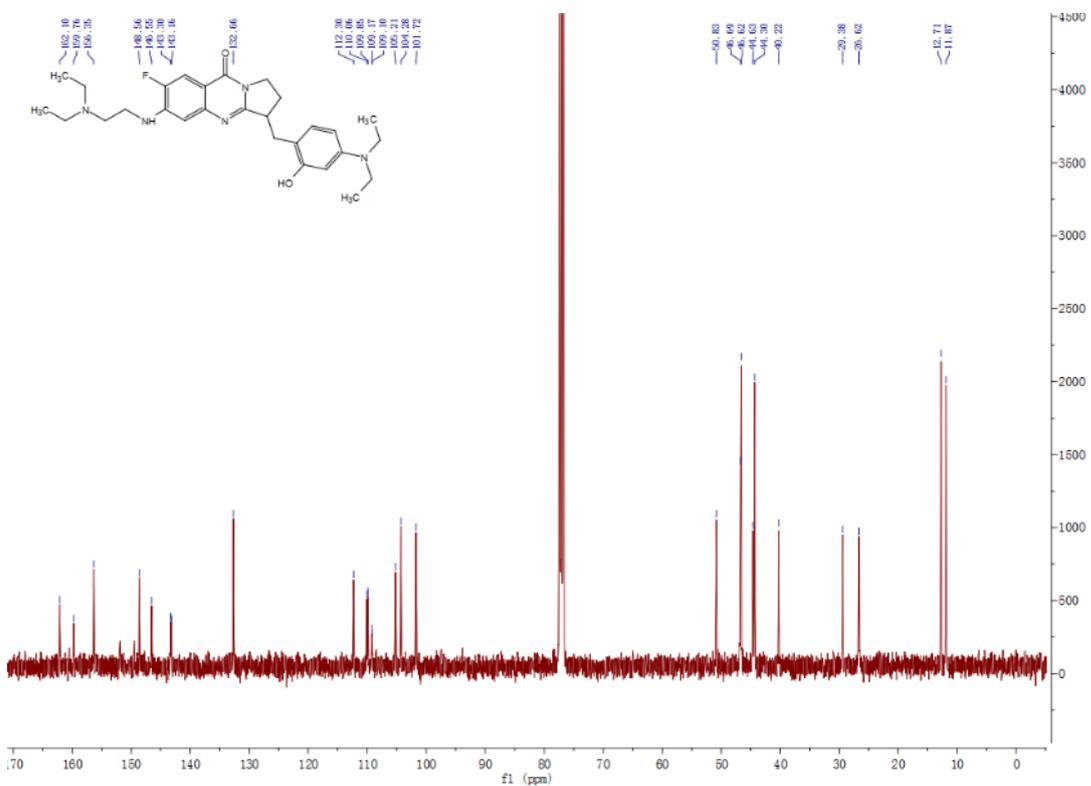


Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Isot	DBE
1	77.92	C26 H34 N5 O F	[M+H] ⁺	452.2817	452.2820	-0.3	-0.66	77.92	12.0

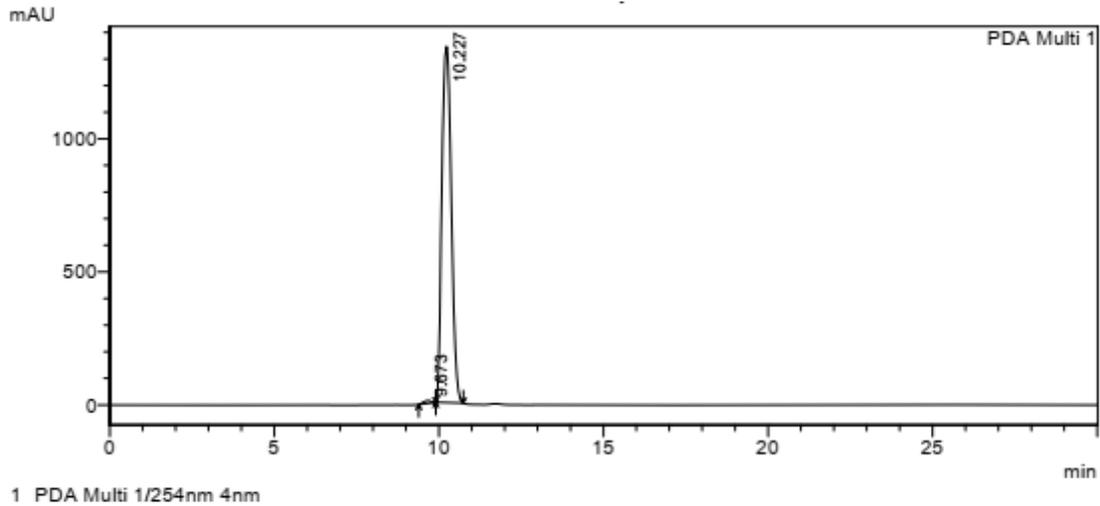
HRMS spectrum of 4g



¹H NMR spectrum of 5g



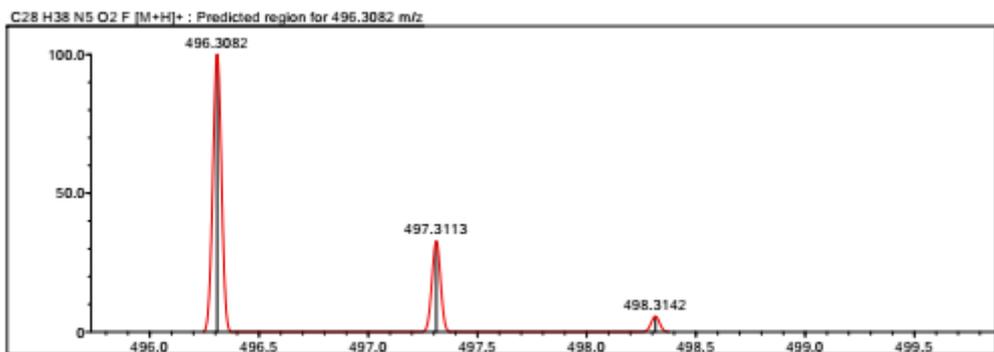
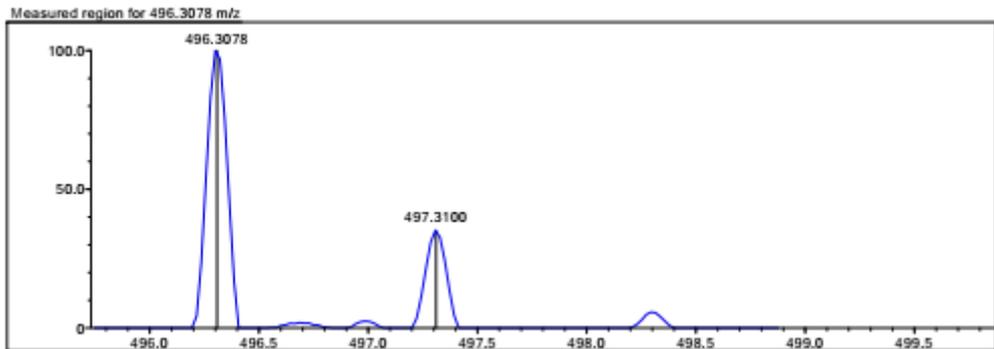
¹³C NMR spectrum of 5g



PeakTable

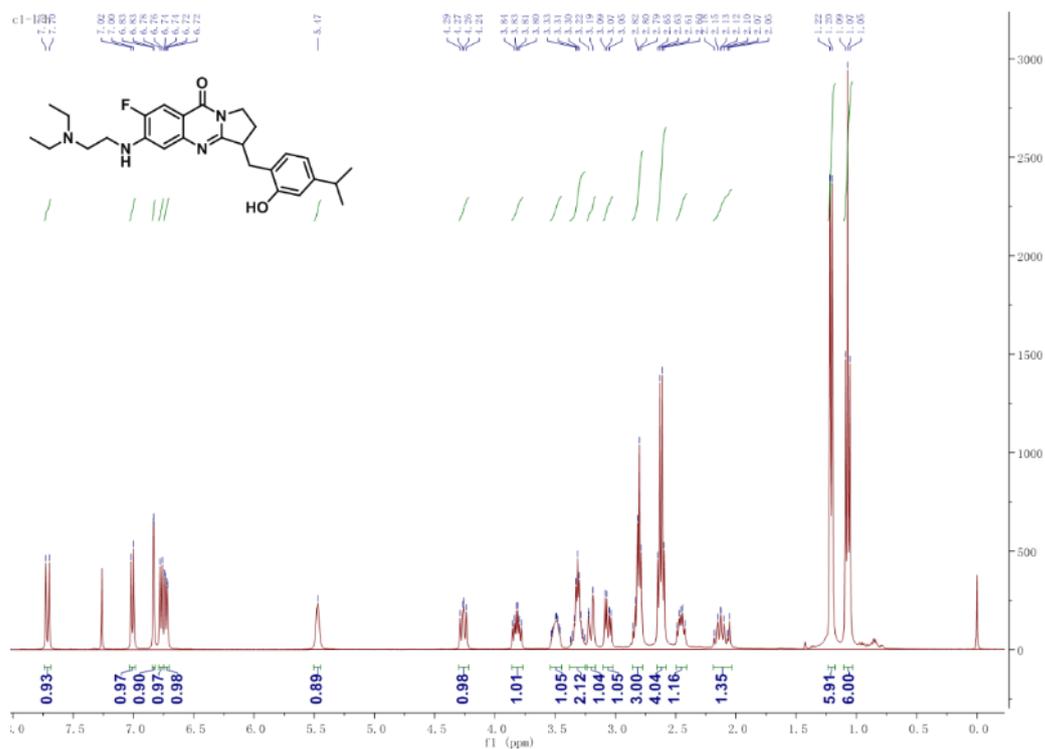
Peak#	Ret. Time	Area	Height	Area %	Height %
1	9.673	155665	10013	0.581	0.743
2	10.227	26647679	1338387	99.419	99.257
Total		26803344	1348400	100.000	100.000

HPLC analysis of 5g

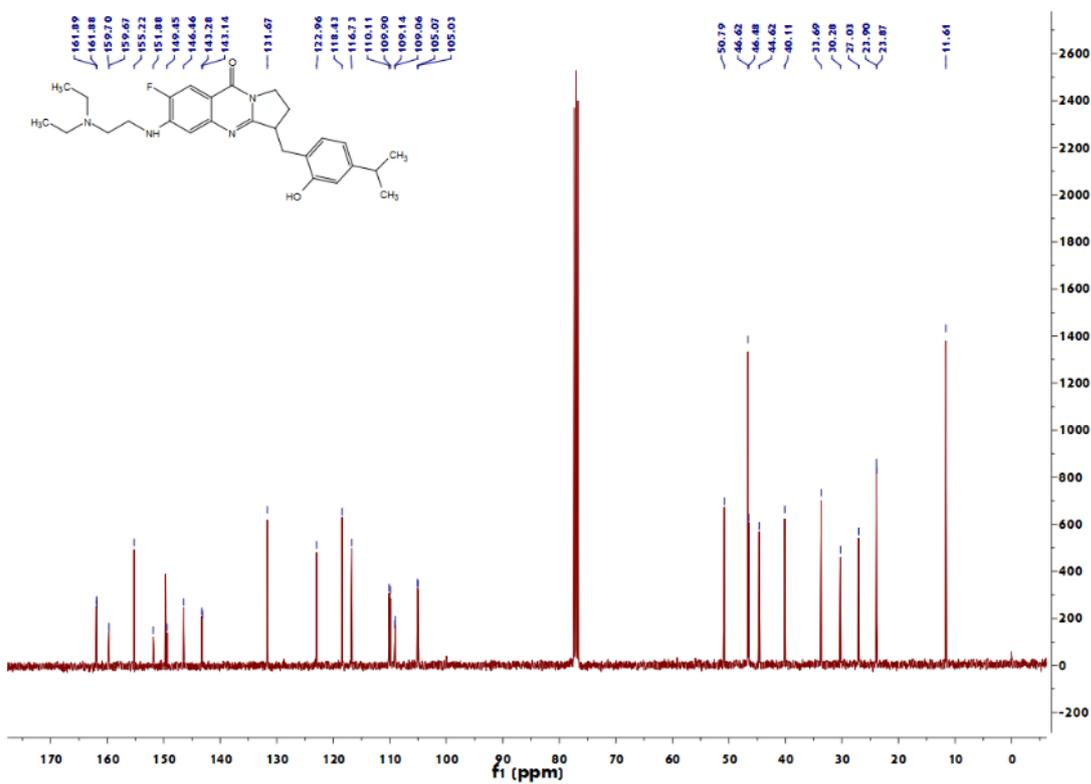


Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Isot	DBE
2	75.17	C28 H38 N5 O2 F	[M+H] ⁺	496.3078	496.3082	-0.4	-0.81	75.17	12.0

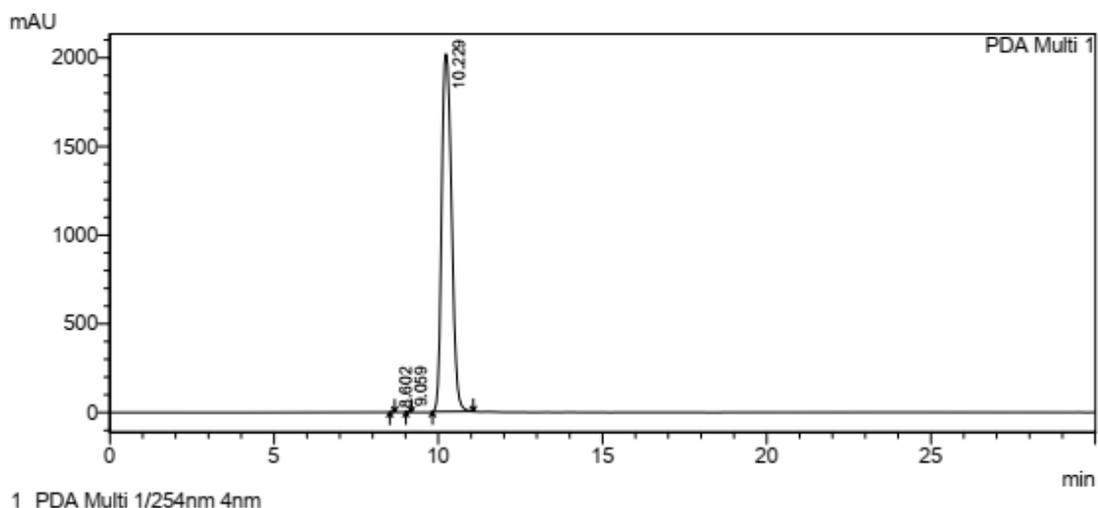
HRMS spectrum of 5g



¹H NMR spectrum of 6g



¹³C NMR spectrum of 6g

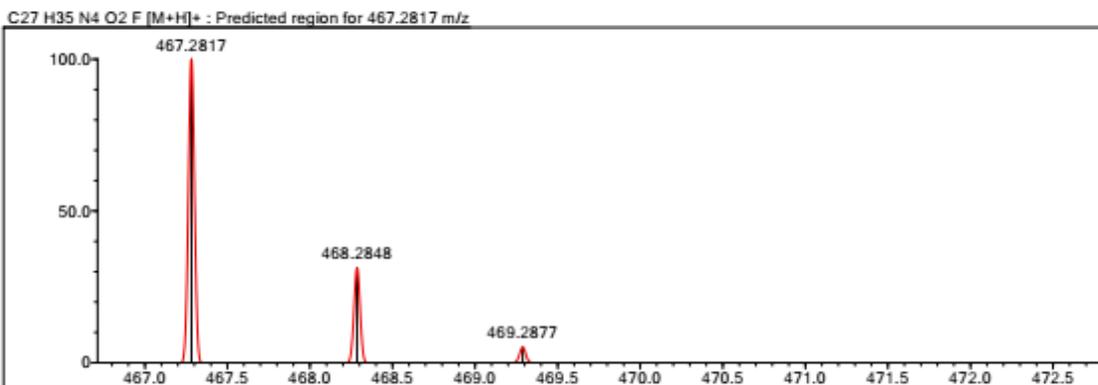
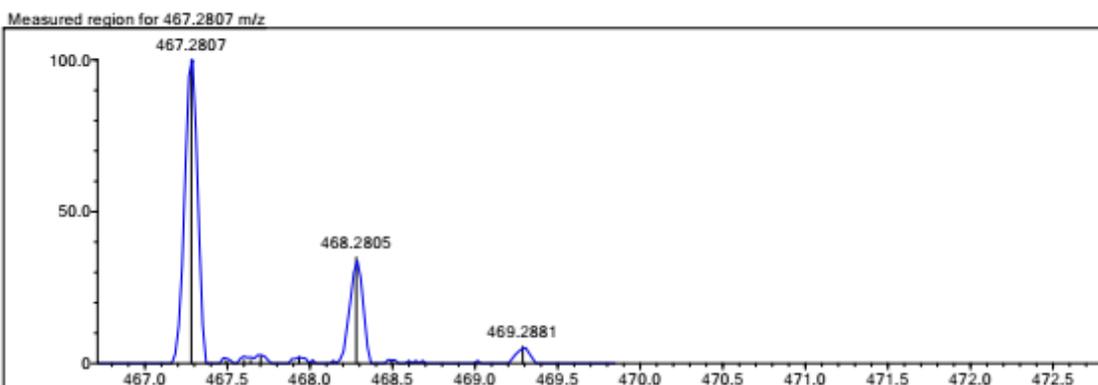


1 PDA Multi 1/254nm 4nm

PeakTable

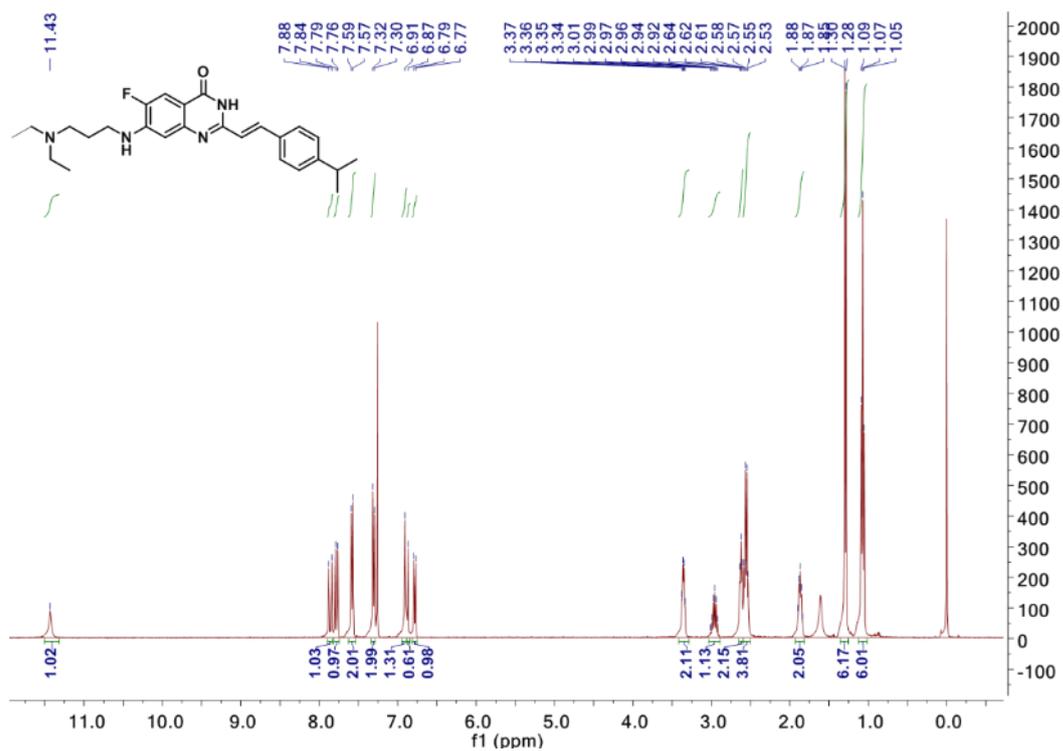
Peak#	Ret. Time	Area	Height	Area %	Height %
1	8.602	3212	525	0.008	0.026
2	9.059	3620	518	0.009	0.026
3	10.229	42261609	2016071	99.984	99.948
Total		42268441	2017113	100.000	100.000

HPLC analysis of **6g**

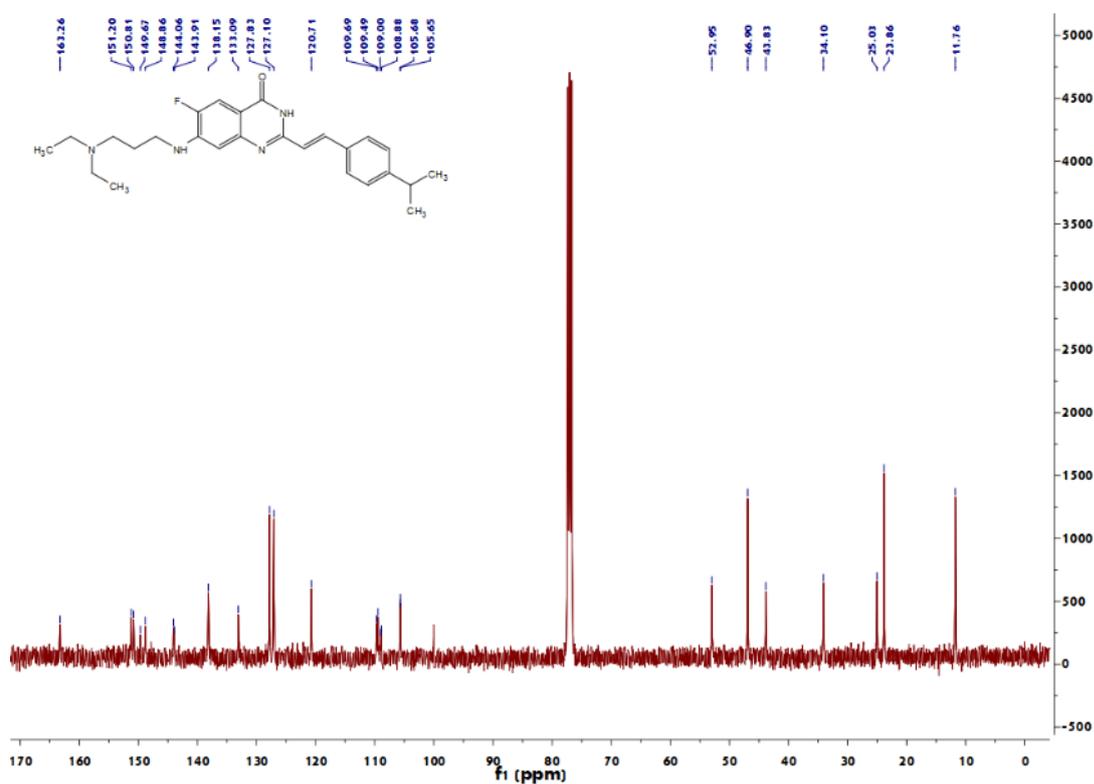


Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
2	78.05	C27 H35 N4 O2 F	[M+H] ⁺	467.2807	467.2817	-1.0	-2.14	80.34	12.0

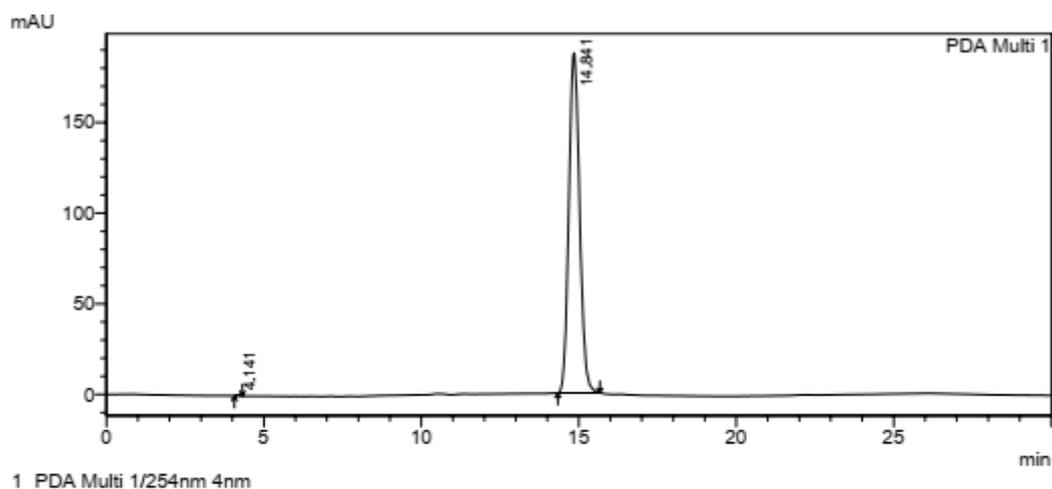
HRMS spectrum of **6g**



¹H NMR spectrum of **1h**



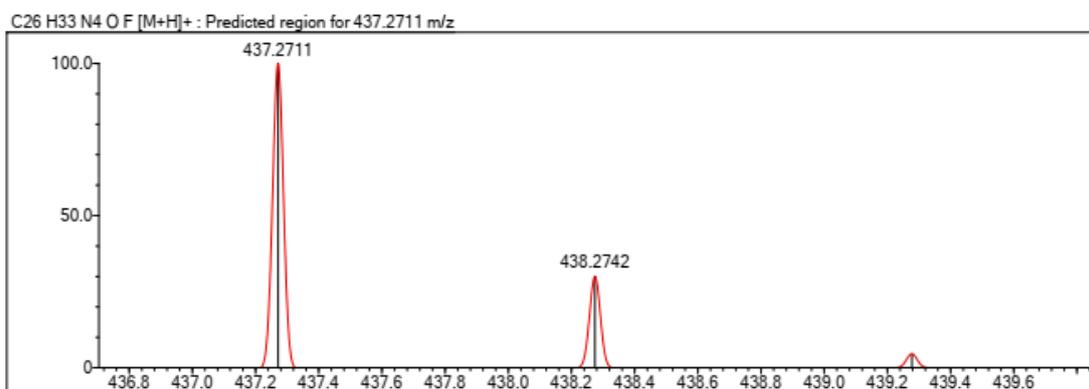
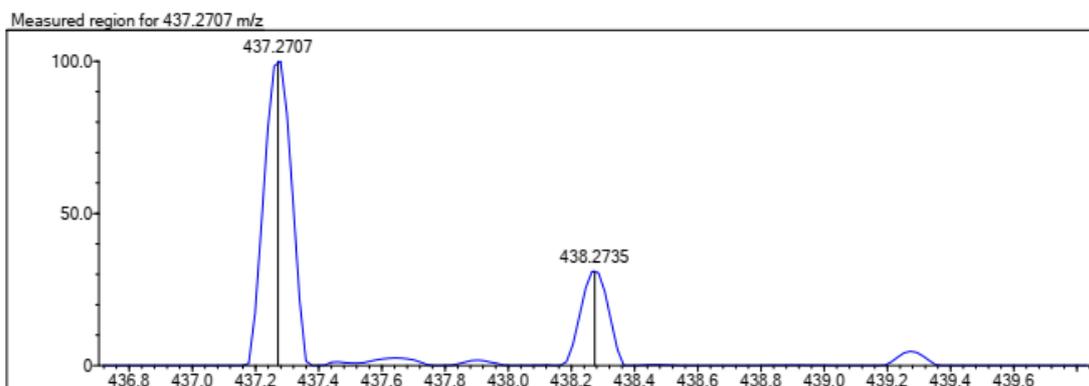
¹³C NMR spectrum of **1h**



PeakTable

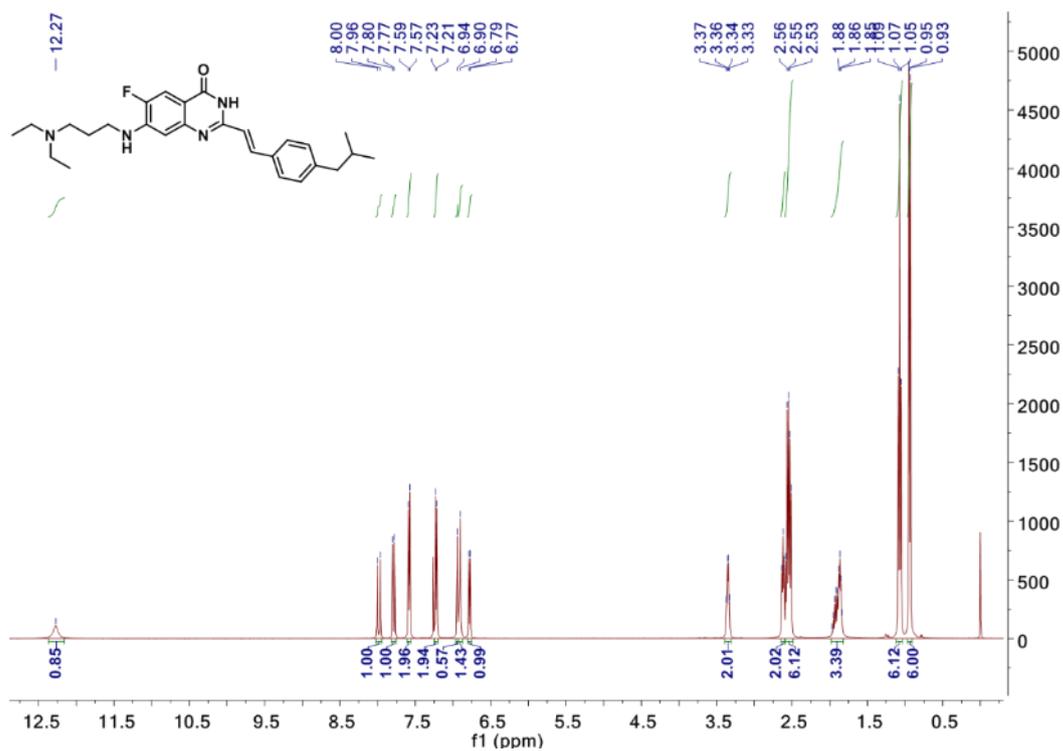
Peak#	Ret. Time	Area	Height	Area %	Height %
1	4.141	5728	588	0.129	0.313
2	14.841	4444600	187525	99.871	99.687
Total		4450327	188113	100.000	100.000

HPLC analysis of 1h

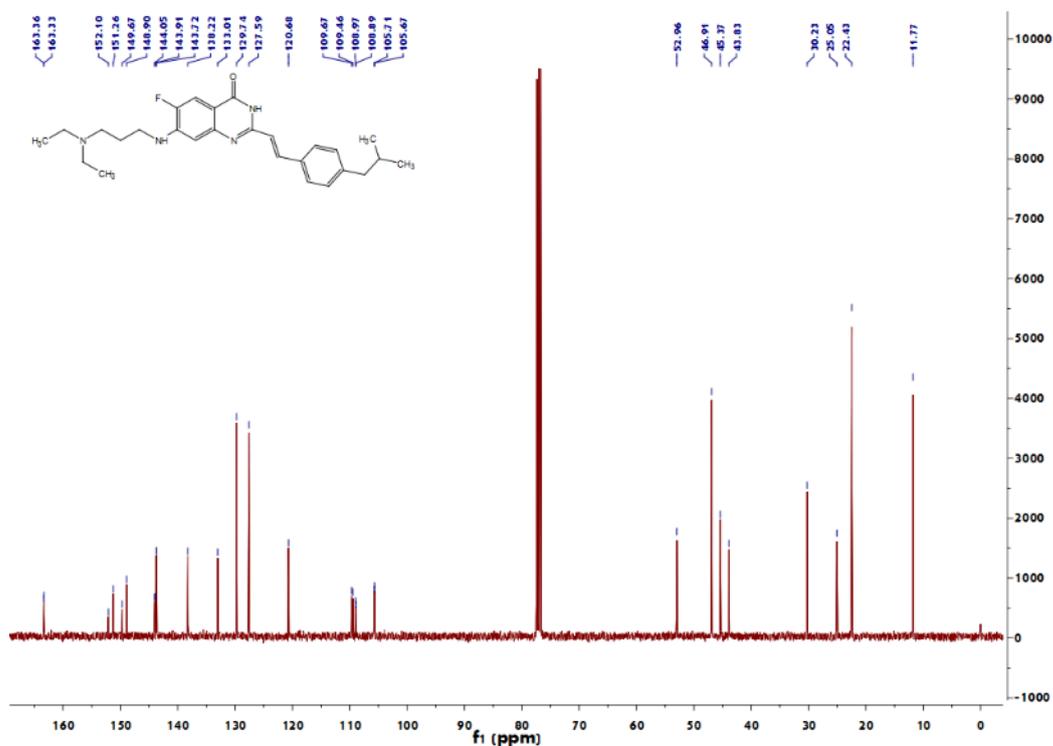


Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Isr	DBE
1	77.72	C26 H33 N4 O F	[M+H] ⁺	437.2707	437.2711	-0.4	-0.91	77.72	12.0

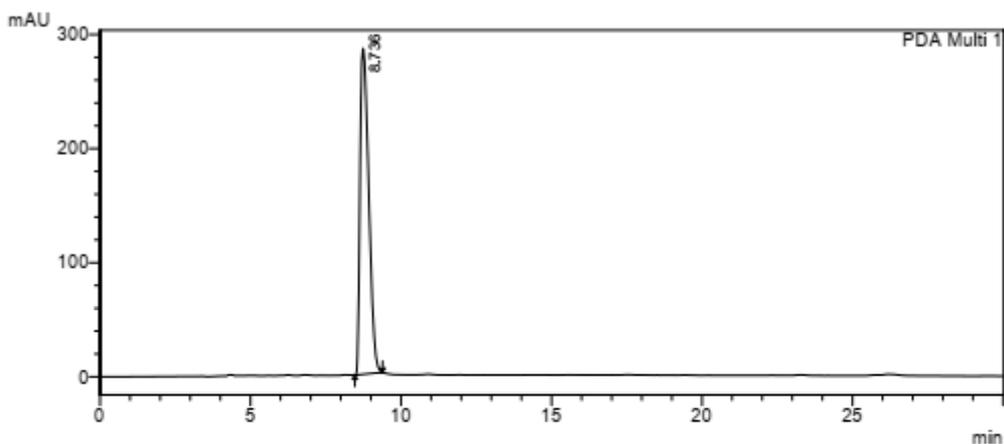
HRMS spectrum of 1h



¹H NMR spectrum of **2h**



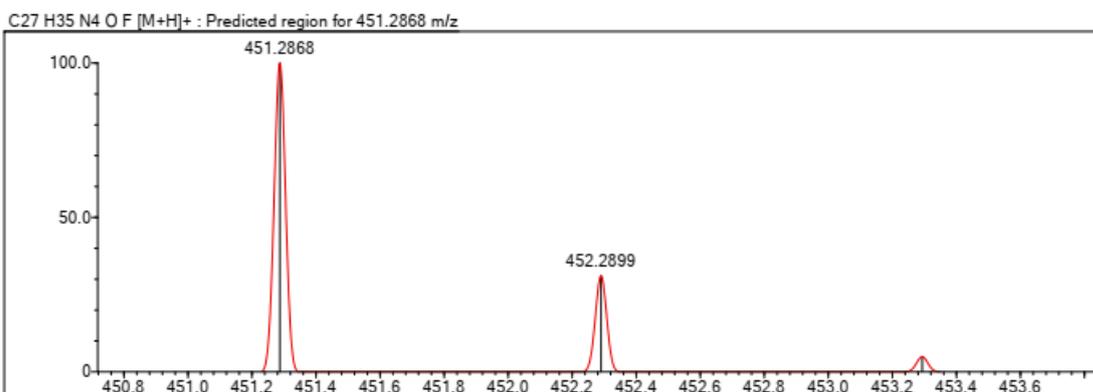
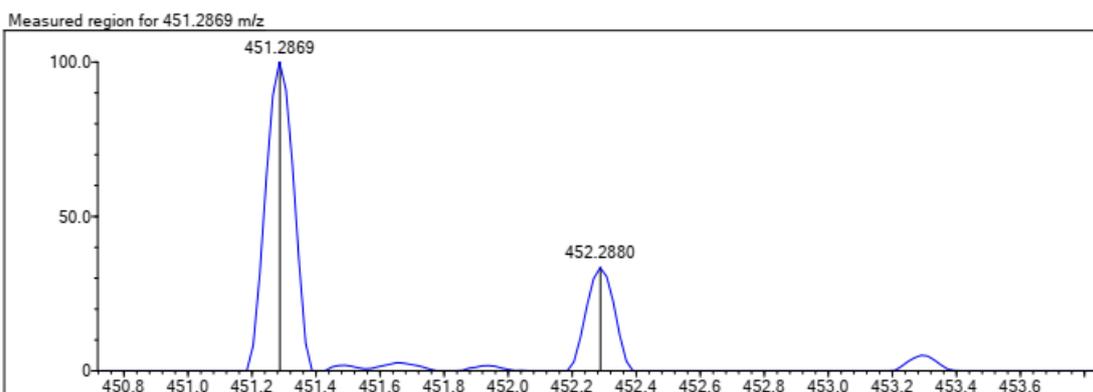
¹³C NMR spectrum of **2h**



PeakTable

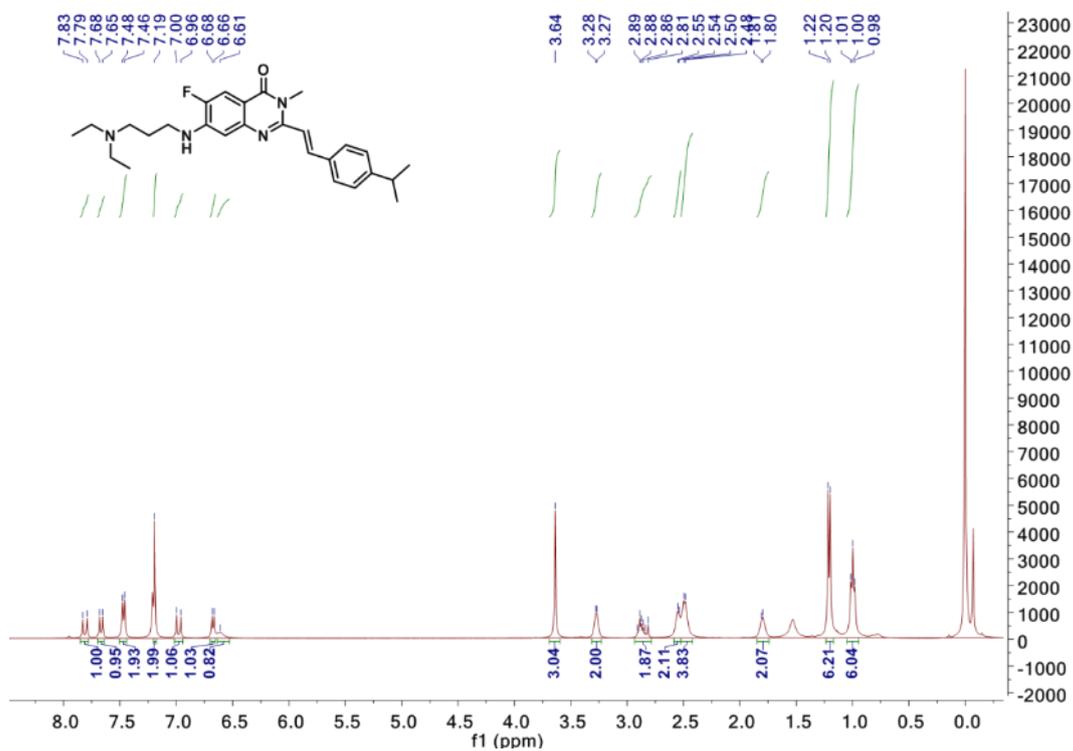
Peak#	Ret. Time	Area	Height	Area %	Height %
1	8.736	5738643	285588	100.000	100.000
Total		5738643	285588	100.000	100.000

HPLC analysis of 2h

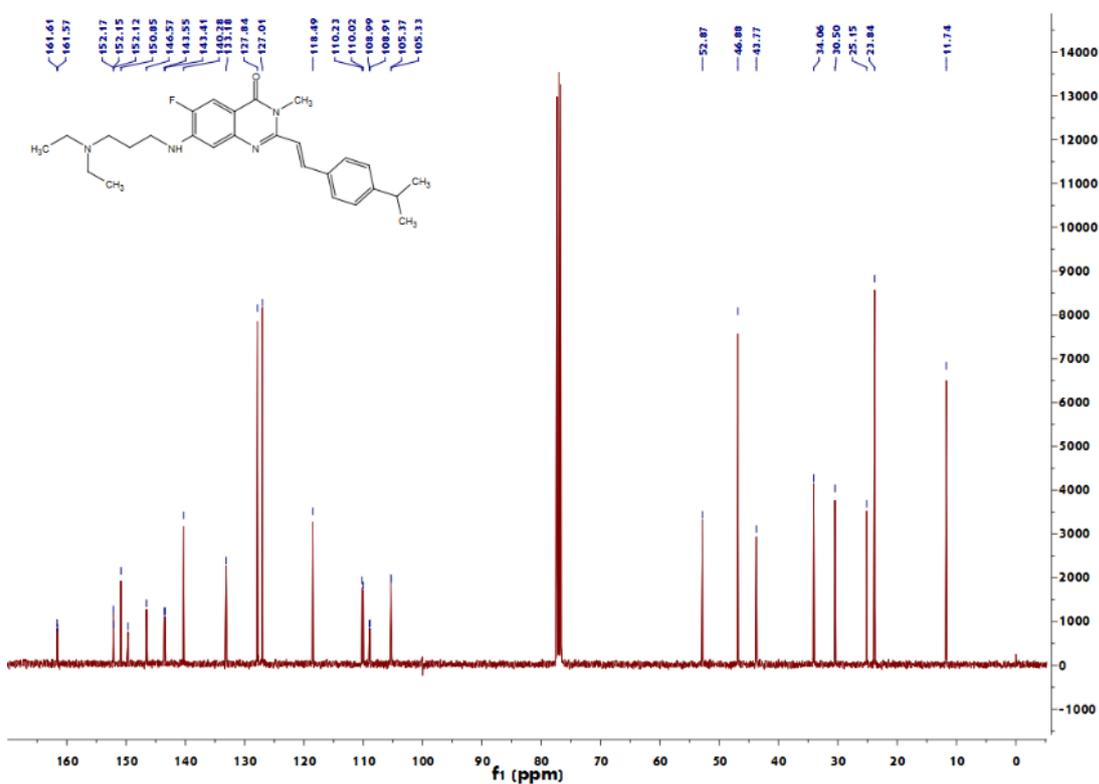


Rank	Score	Formula (M)	Ion	Mess. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	79.53	C27 H35 N4 O F	[M+H] ⁺	451.2869	451.2868	0.1	0.22	79.53	12.0

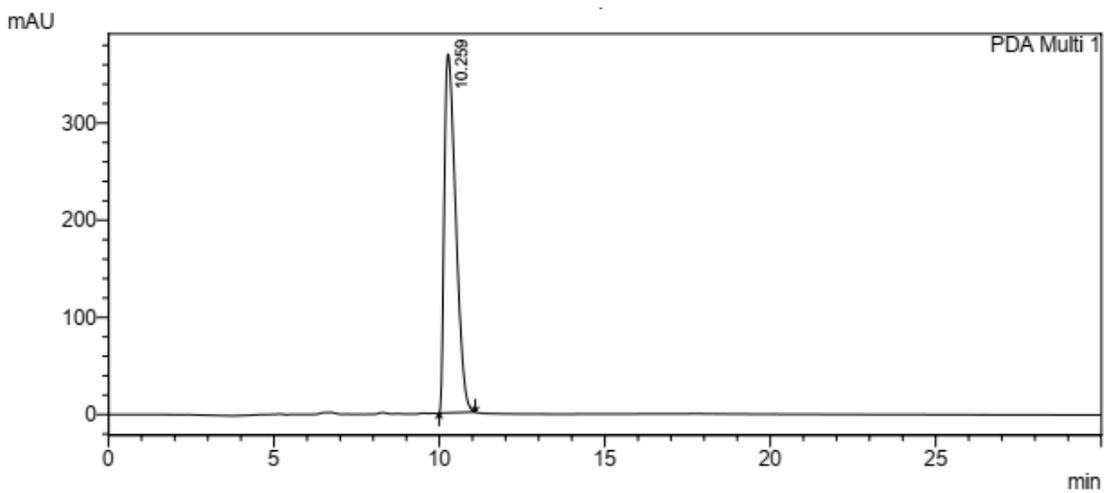
HRMS spectrum of 2h



¹H NMR spectrum of 3h



¹³C NMR spectrum of 3h



1 PDA Multi 1/254nm 4nm

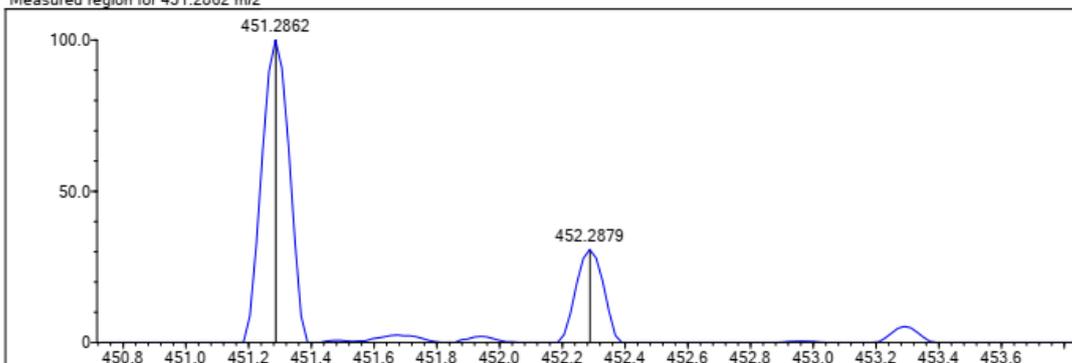
PeakTable

PDA Ch1 254nm 4nm

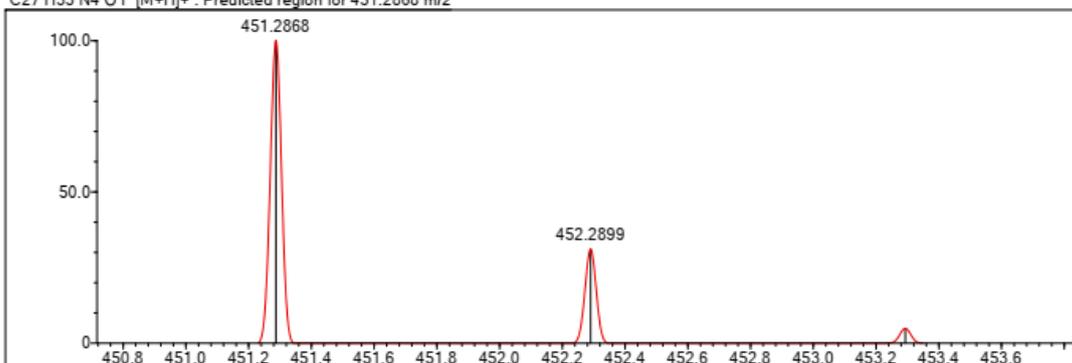
Peak#	Ret. Time	Area	Height	Area %	Height %
1	10.259	8898534	369249	100.000	100.000
Total		8898534	369249	100.000	100.000

HPLC analysis of 3h

Measured region for 451.2862 m/z

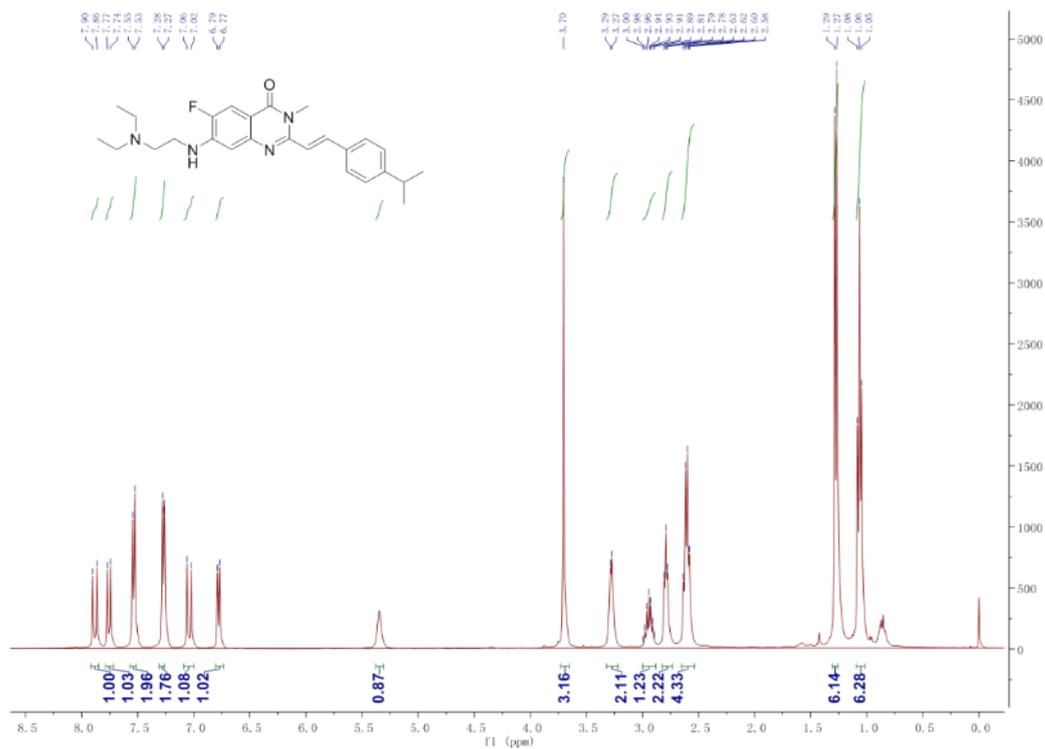


C27 H35 N4 O F [M+H]⁺ : Predicted region for 451.2868 m/z

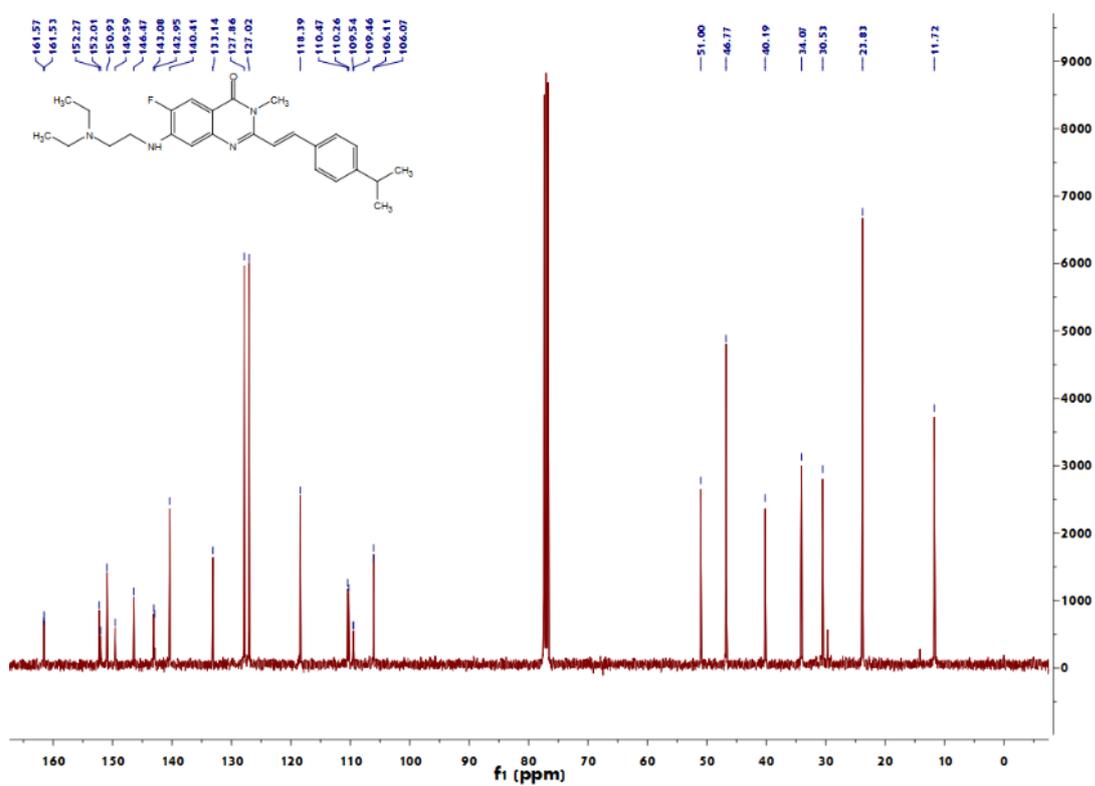


Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
3	79.06	C27 H35 N4 O F	[M+H] ⁺	451.2862	451.2868	-0.6	-1.33	79.72	12.0

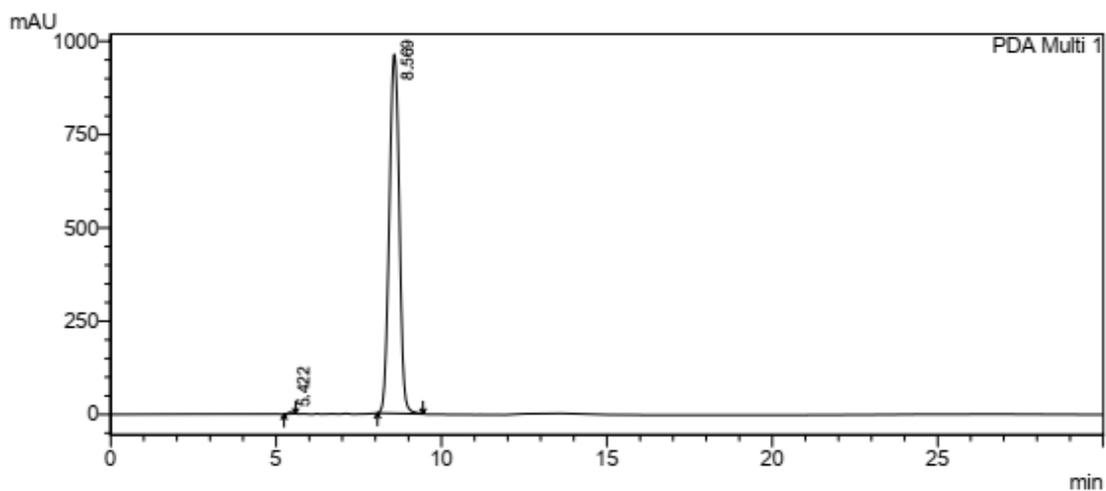
HRMS spectrum of 3h



¹H NMR spectrum of 4h



¹³C NMR spectrum of 4h

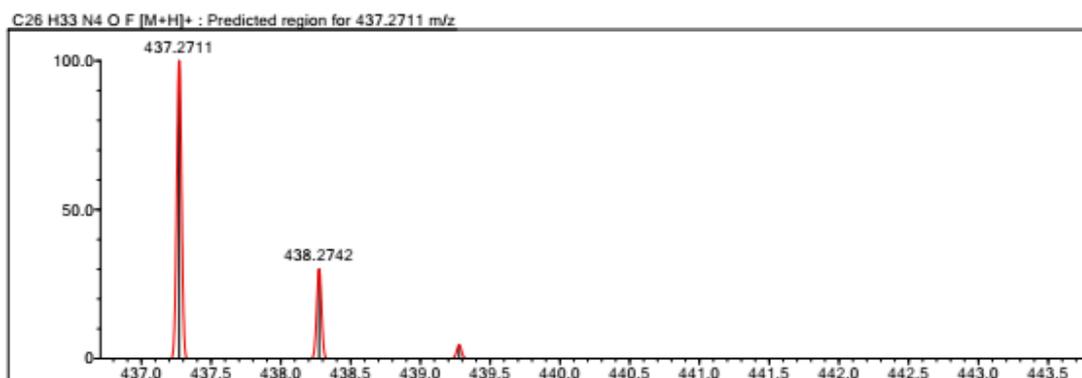
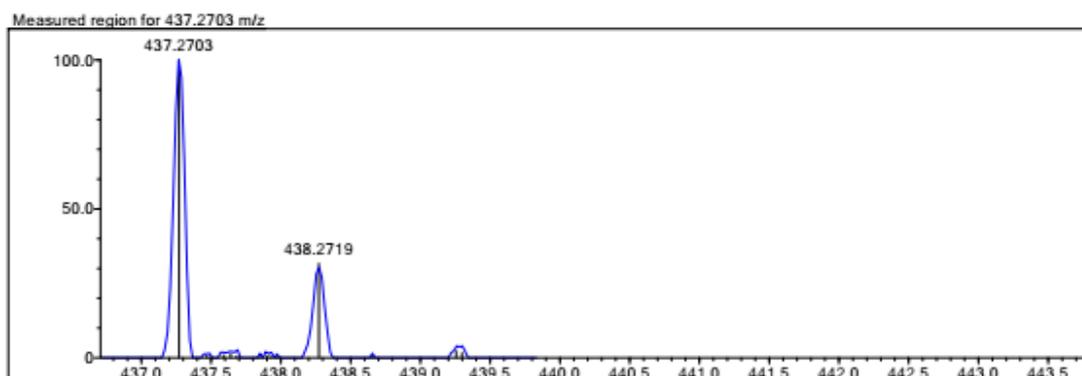


1 PDA Multi 1/254nm 4nm

PeakTable

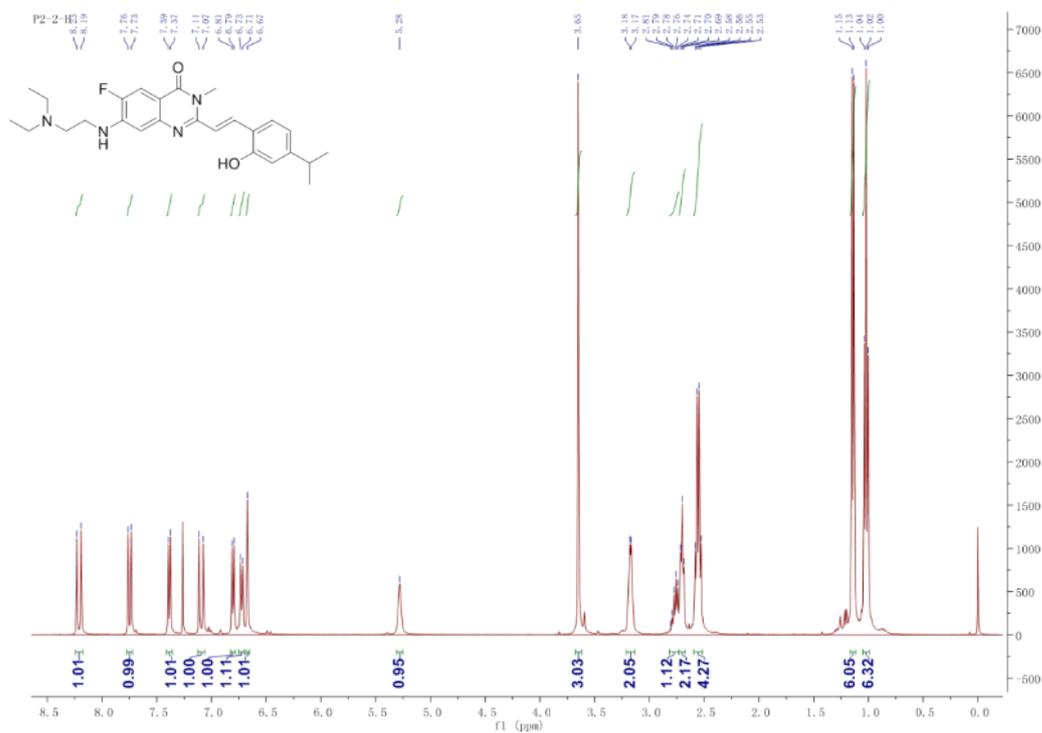
Peak#	Ret. Time	Area	Height	Area %	Height %
1	5.422	44866	4634	0.221	0.479
2	8.569	20215773	962216	99.779	99.521
Total		20260639	966850	100.000	100.000

HPLC analysis of 4h

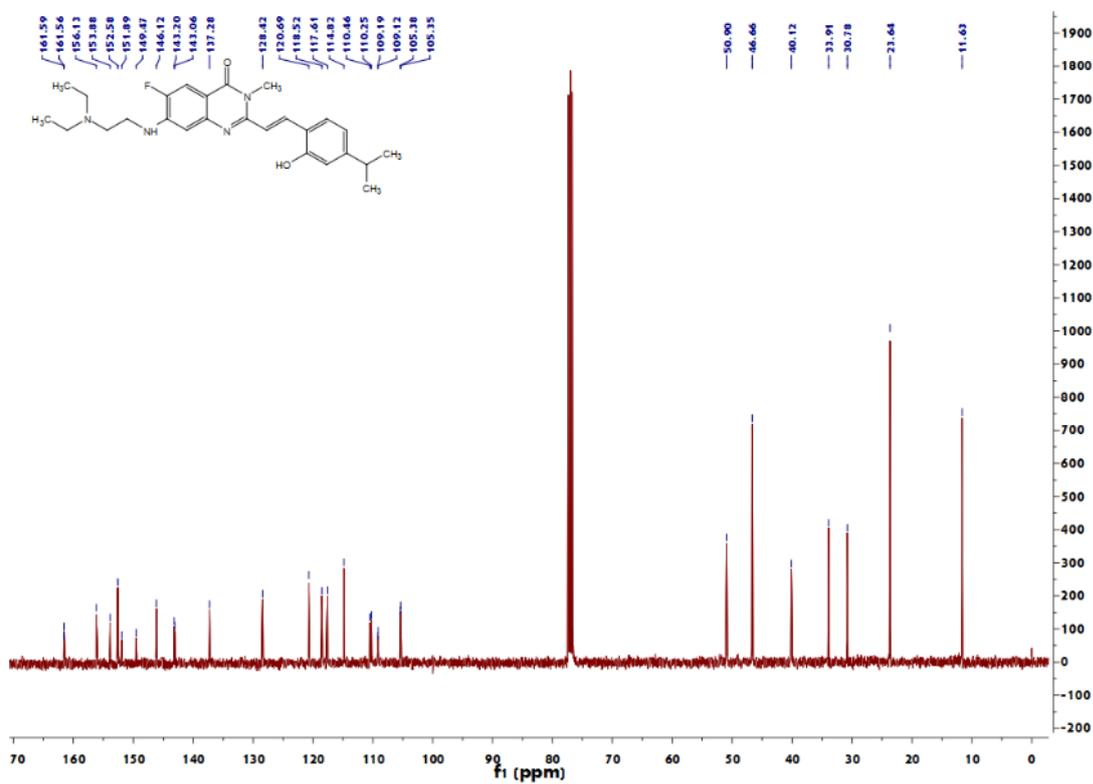


Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Isot	DBE
2	93.22	C ₂₆ H ₃₃ N ₄ O F	[M+H] ⁺	437.2703	437.2711	-0.8	-1.83	95.20	12.0

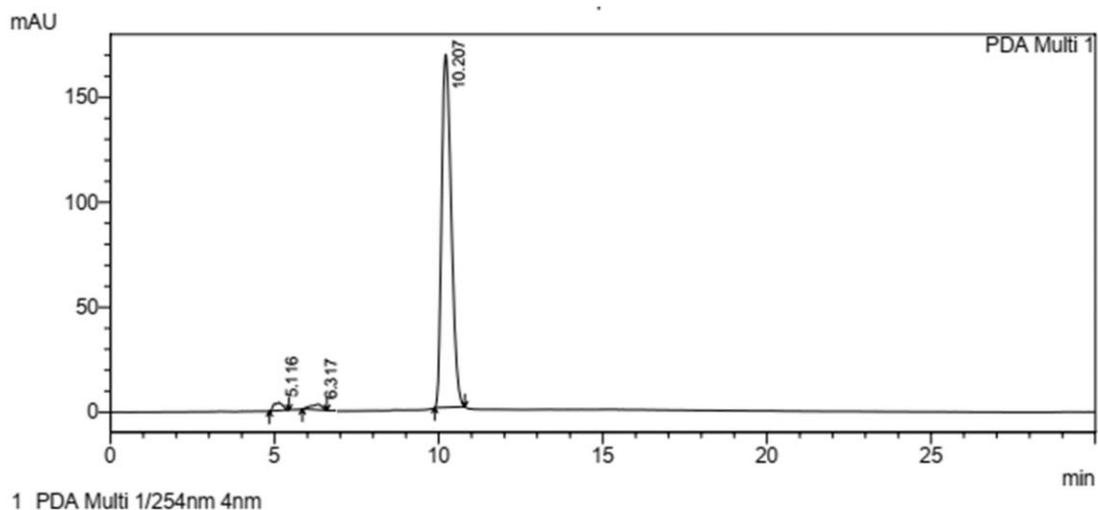
HRMS spectrum of 4h



¹H NMR spectrum of 5h



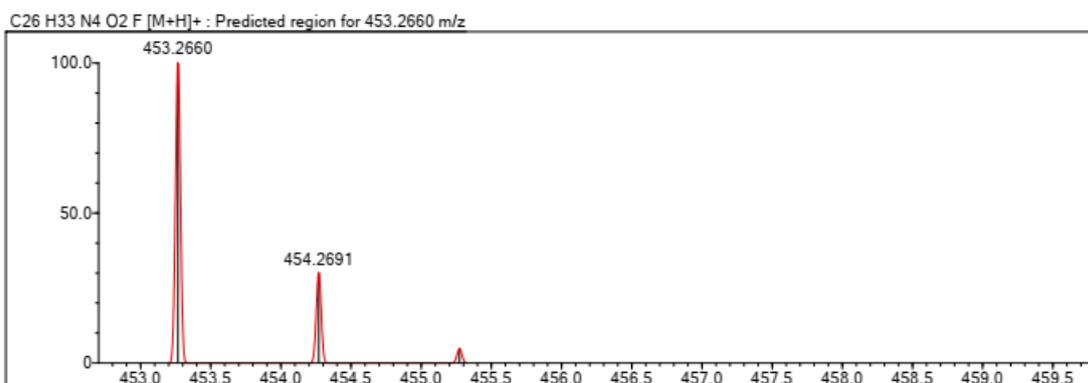
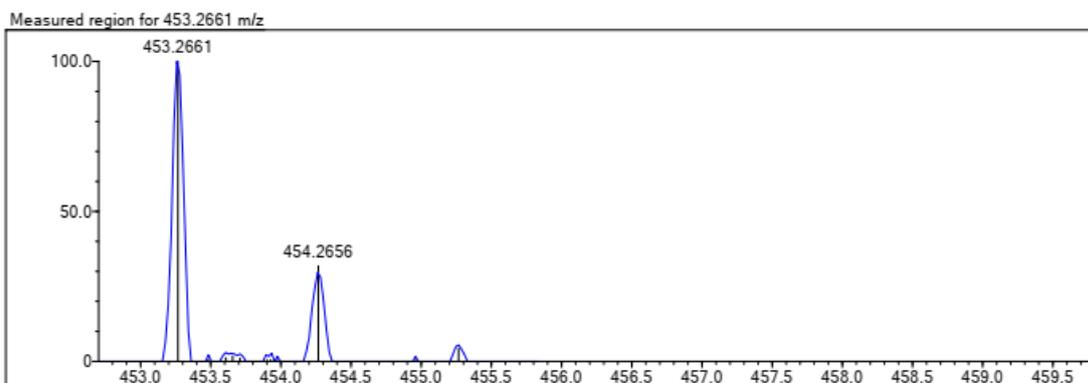
¹³C NMR spectrum of 5h



PeakTable

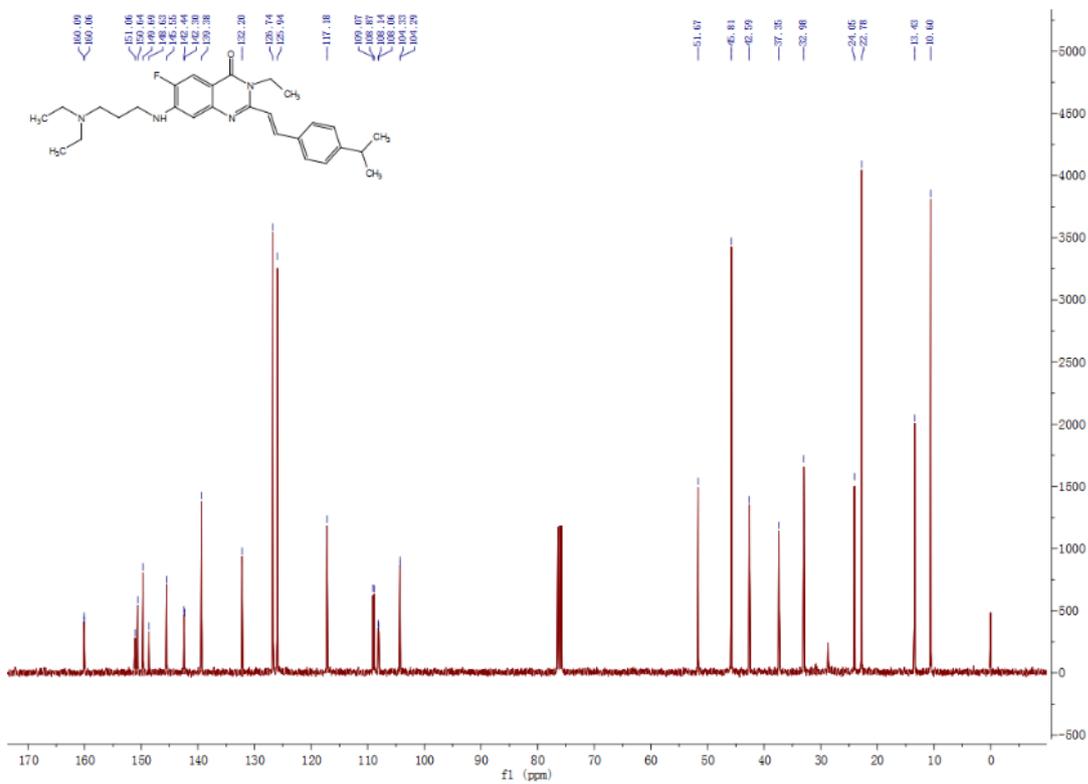
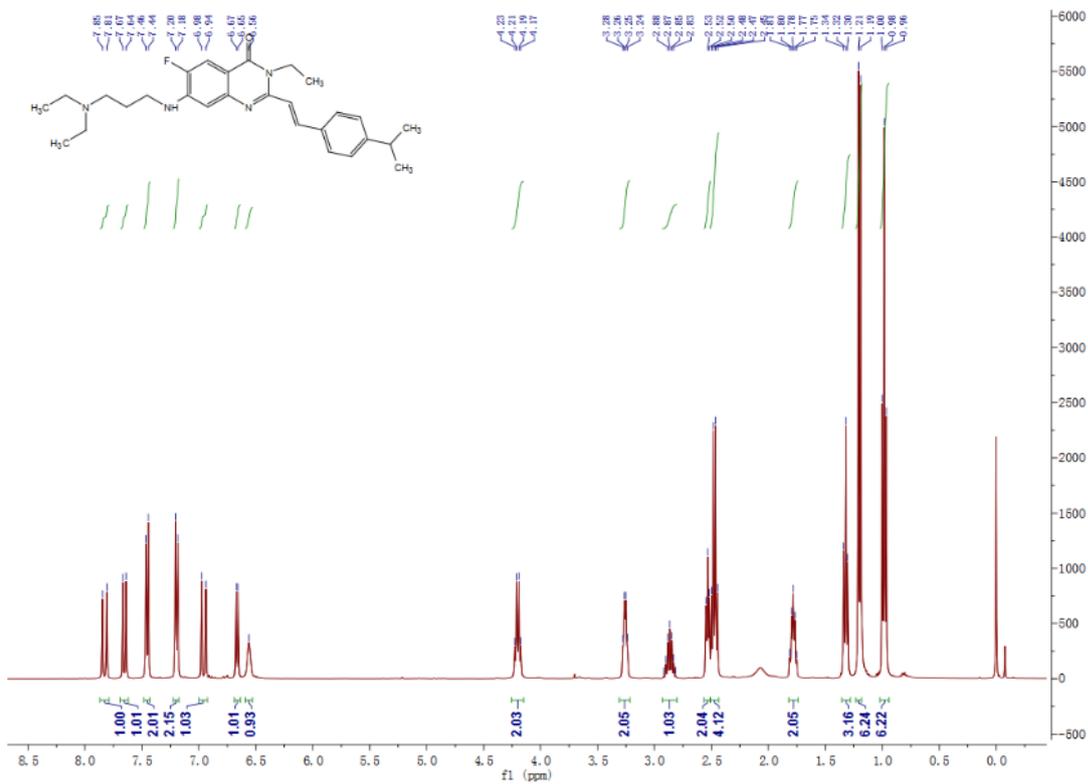
Peak#	Ret. Time	Area	Height	Area %	Height %
1	5.116	71050	3765	1.996	2.157
2	6.317	61474	2619	1.727	1.500
3	10.207	3426587	168190	96.276	96.343
Total		3559111	174574	100.000	100.000

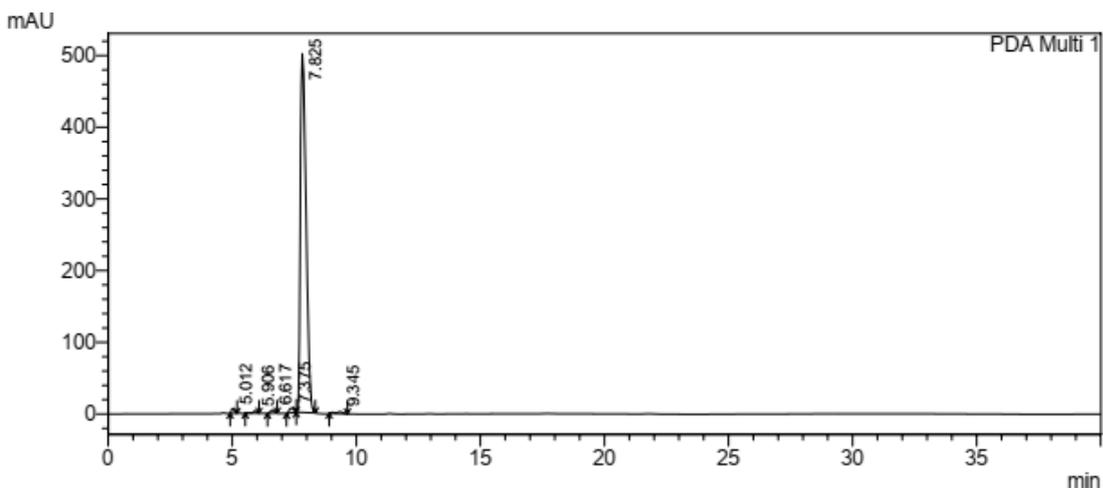
HPLC analysis of 5h



Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	86.14	C26H33N4O2F	[M+H] ⁺	453.2661	453.2660	0.1	0.22	86.14	12.0

HRMS spectrum of 5h





1 PDA Multi 1/254nm 4nm

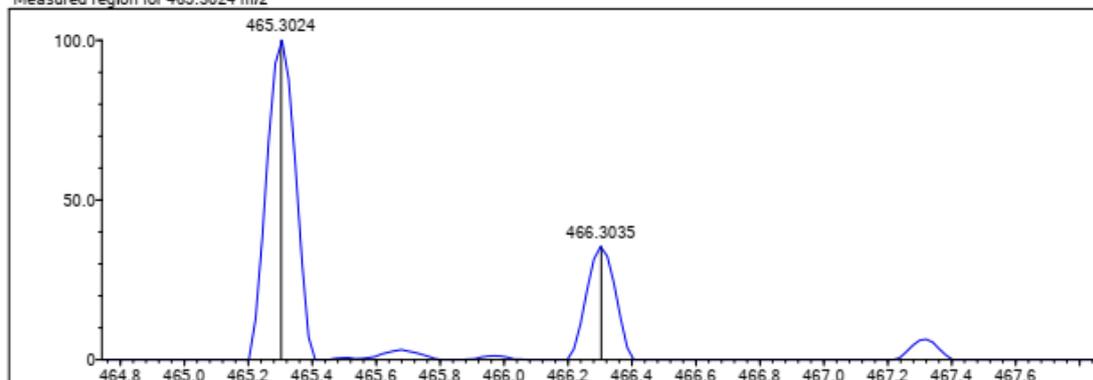
PeakTable

PDA Ch1 254nm 4nm

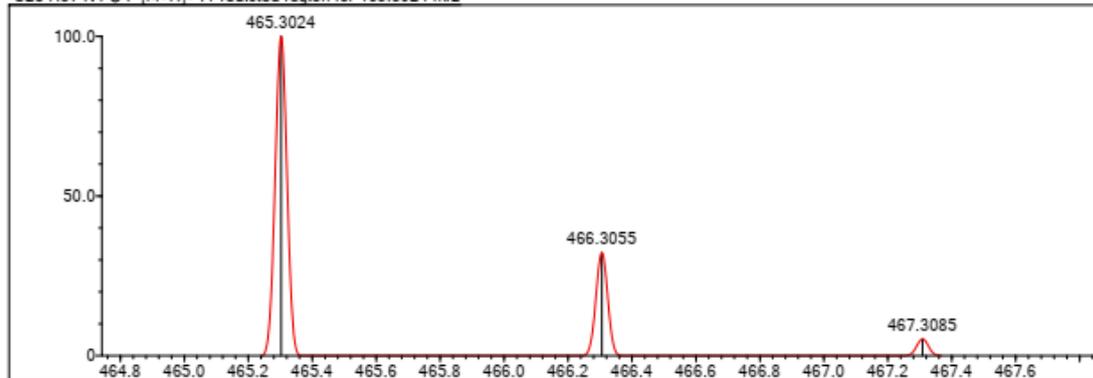
Peak#	Ret. Time	Area	Height	Area %	Height %
1	5.012	56750	6393	0.689	1.222
2	5.906	29299	2821	0.356	0.539
3	6.617	52639	4394	0.639	0.840
4	7.375	79899	6486	0.970	1.240
5	7.825	7961497	500398	96.639	95.656
6	9.345	58335	2630	0.708	0.503
Total		8238418	523121	100.000	100.000

HPLC analysis of 6h

Measured region for 465.3024 m/z

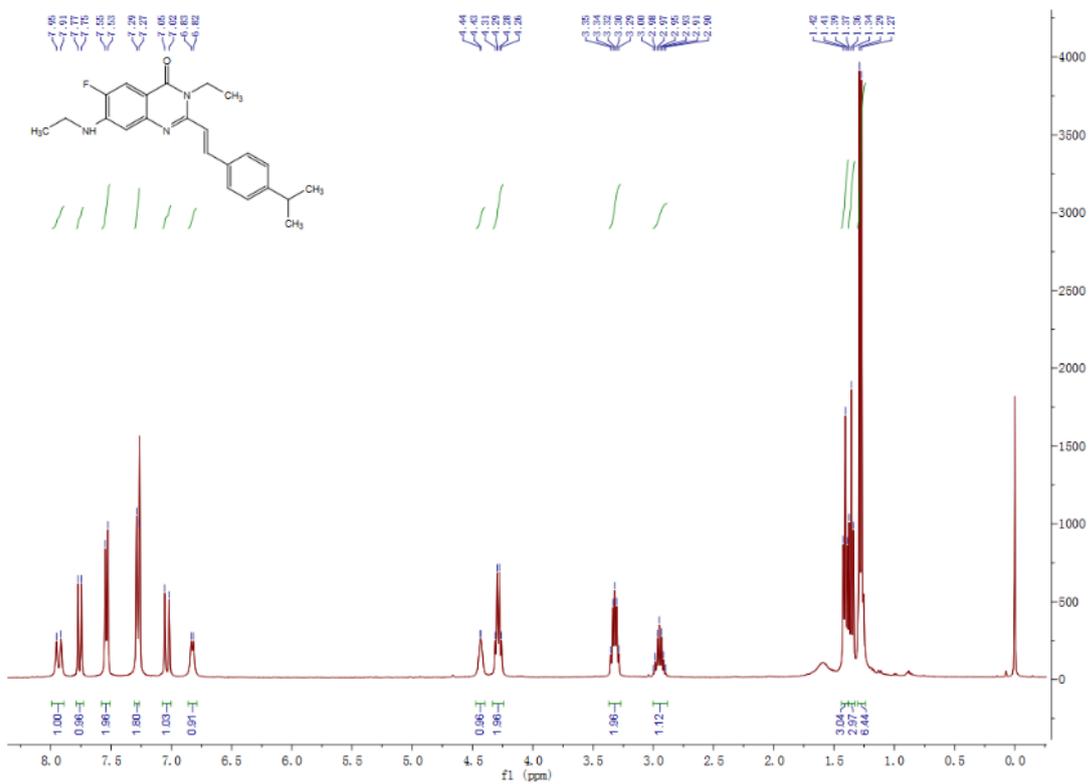


C28 H37 N4 O F [M+H]⁺ : Predicted region for 465.3024 m/z

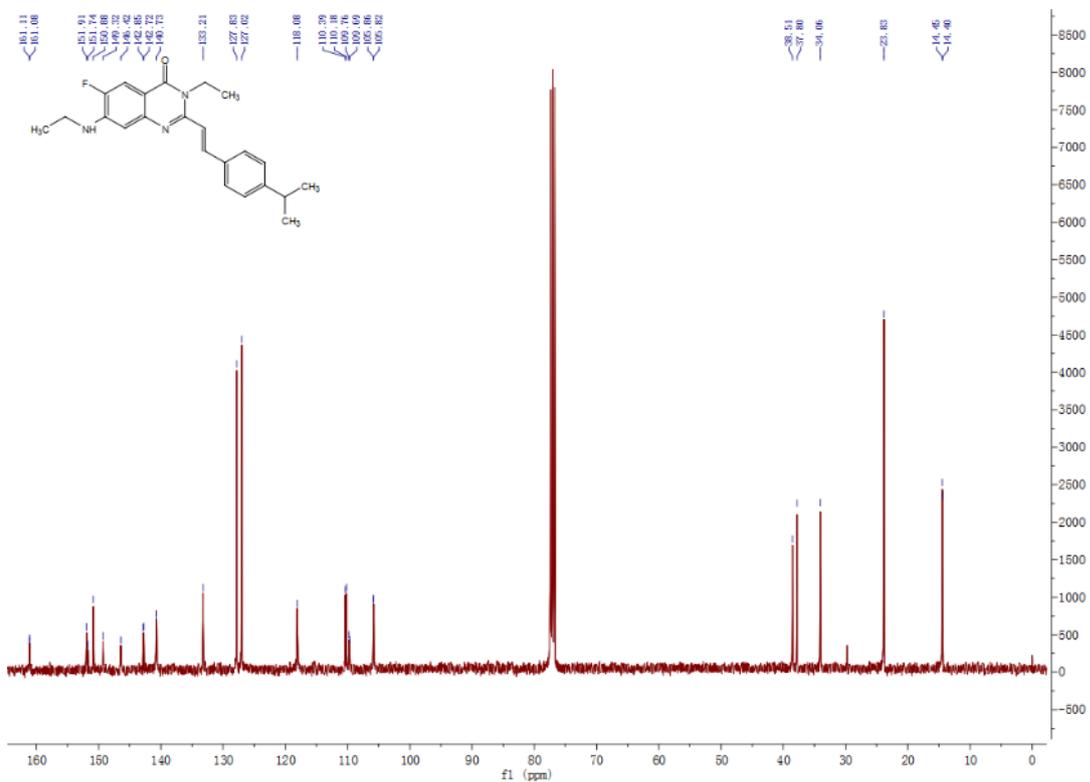


Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Isot	DBE
1	91.06	C28 H37 N4 O F	[M+H] ⁺	465.3024	465.3024	-0.0	0.00	91.06	12.0

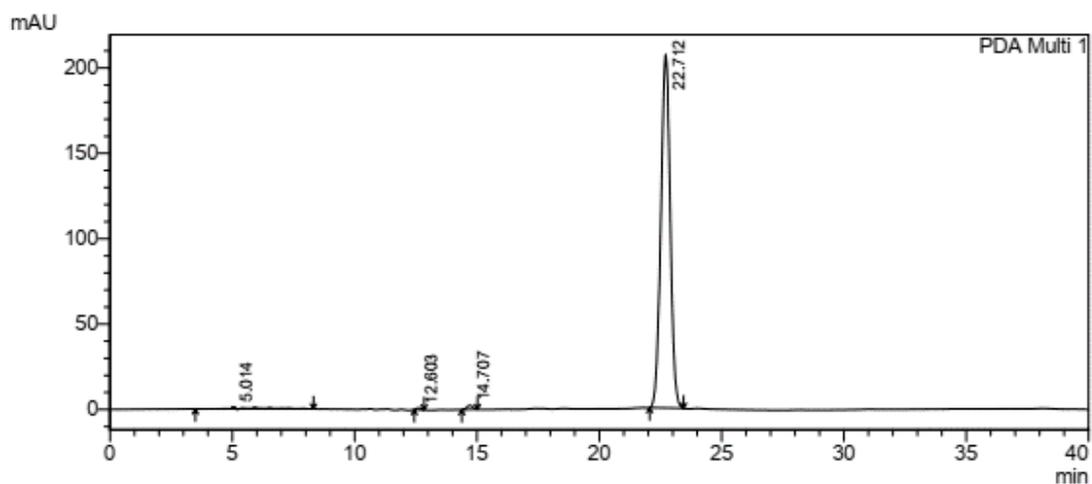
HRMS spectrum of 6h



¹H NMR spectrum of 7h



¹³C NMR spectrum of 7h



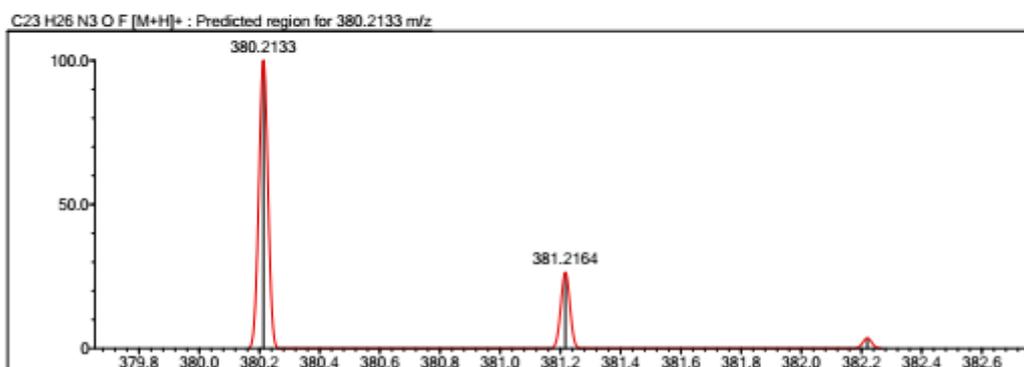
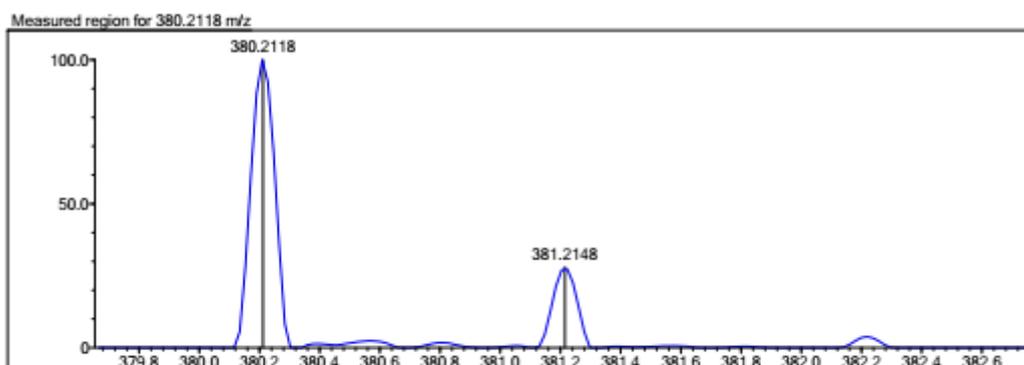
1 PDA Multi 1/254nm 4nm

PeakTable

PDA Ch1 254nm 4nm

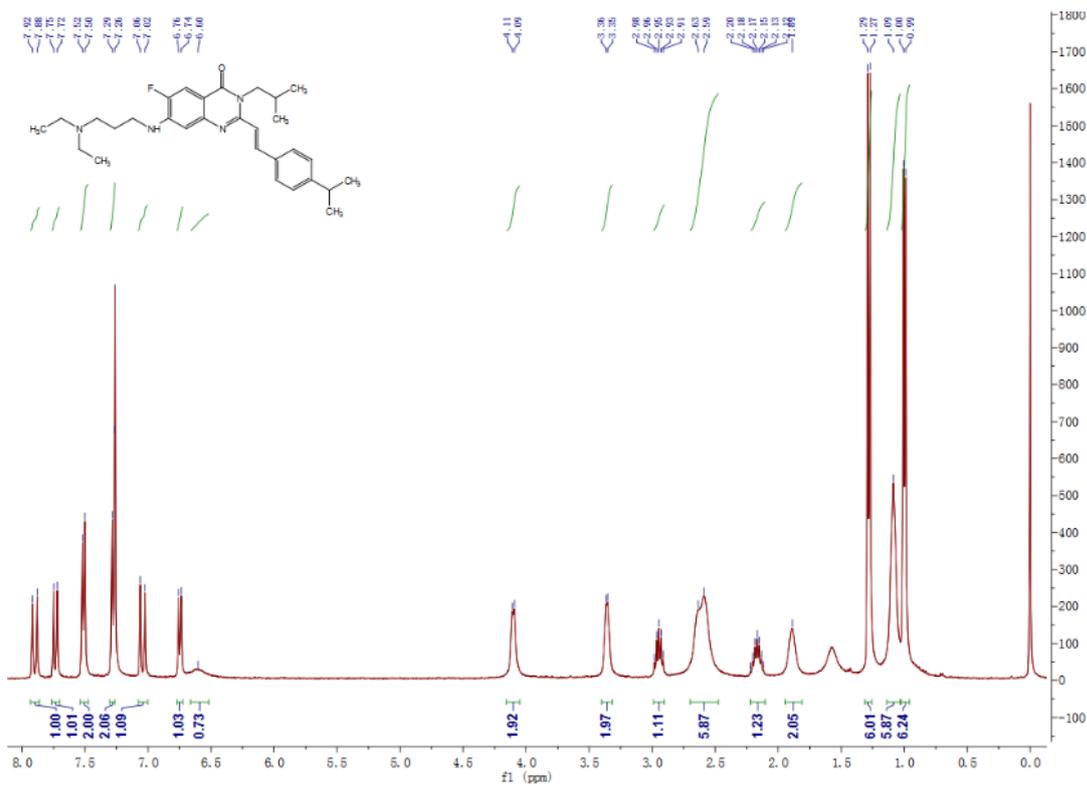
Peak#	Ret. Time	Area	Height	Area %	Height %
1	5.014	76706	1233	1.370	0.583
2	12.603	13063	987	0.233	0.467
3	14.707	41075	2355	0.734	1.113
4	22.712	5467184	206938	97.663	97.837
Total		5598029	211513	100.000	100.000

HPLC analysis of 7h

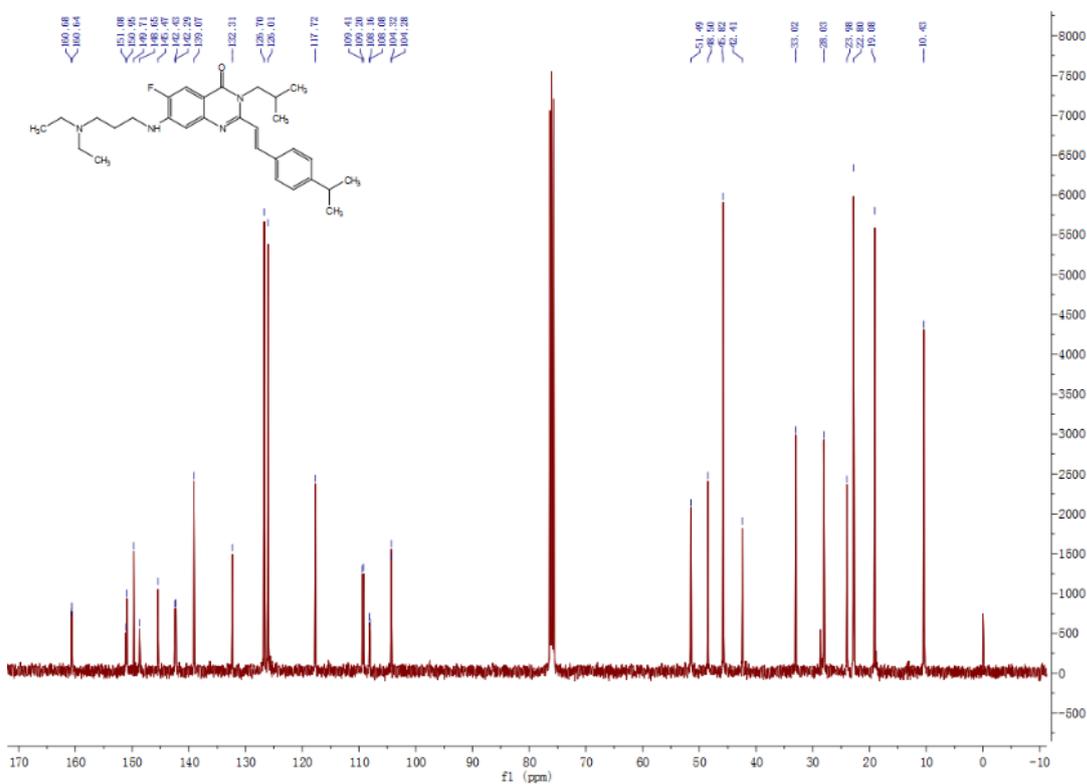


Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Isc	DBE
3	76.04	C ₂₃ H ₂₆ N ₃ O ₂ F	[M+H] ⁺	380.2118	380.2133	-1.5	-3.95	82.10	12.0

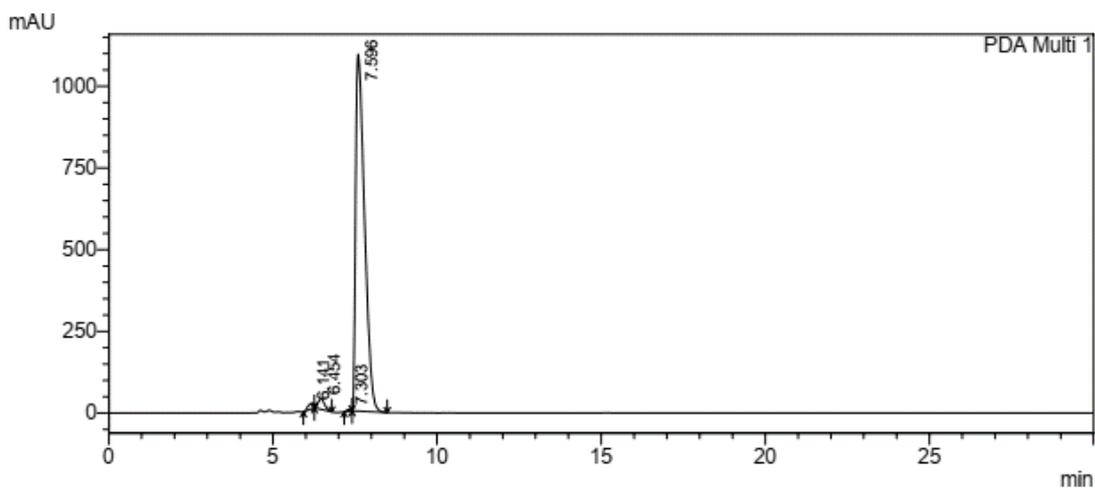
HRMS spectrum of 7h



¹H NMR spectrum of 8h



¹³C NMR spectrum of 8h



1 PDA Multi 1/254nm 4nm

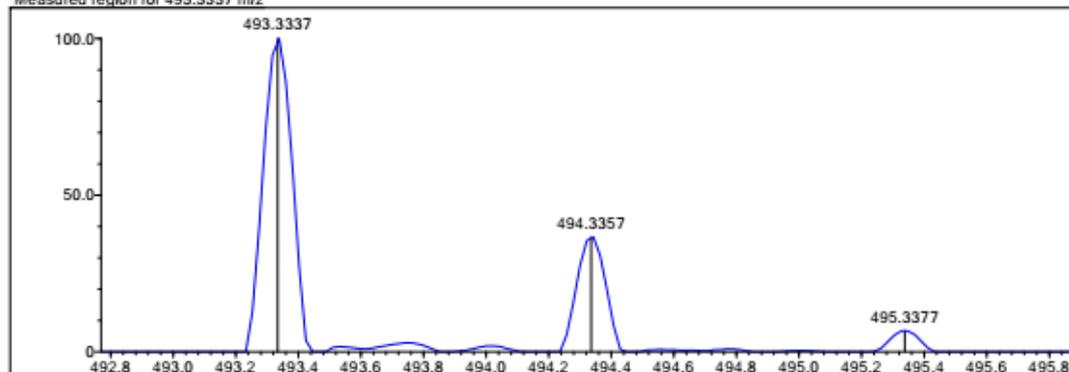
PeakTable

PDA Ch1 254nm 4nm

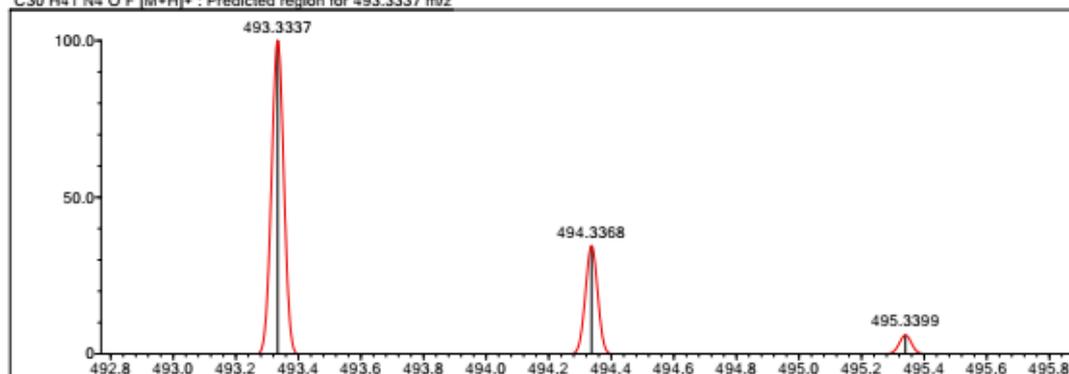
Peak#	Ret. Time	Area	Height	Area %	Height %
1	6.141	163262	14920	0.772	1.305
2	6.454	413844	30968	1.957	2.709
3	7.303	47325	5824	0.224	0.509
4	7.596	20521471	1091619	97.047	95.477
Total		21145902	1143331	100.000	100.000

HPLC analysis of **8h**

Measured region for 493.3337 m/z

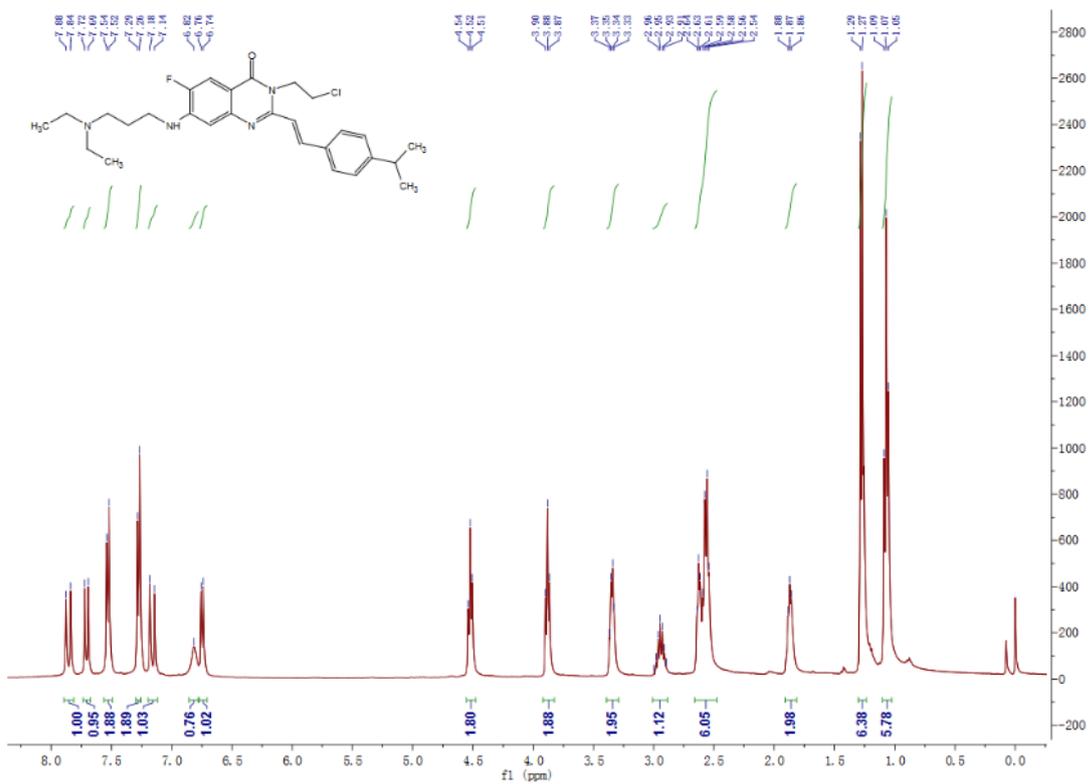


C30 H41 N4 O F [M+H]⁺ : Predicted region for 493.3337 m/z

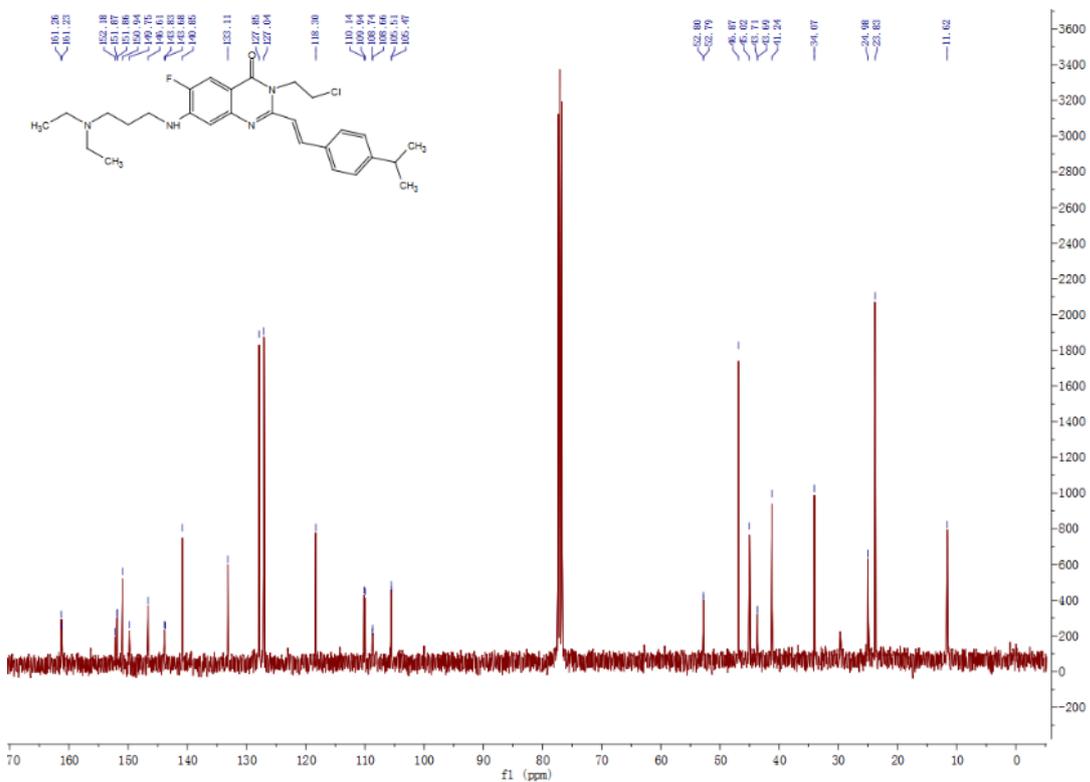


Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Isot	DBE
1	85.95	C30 H41 N4 O F	[M+H] ⁺	493.3337	493.3337	-0.0	0.00	85.95	12.0

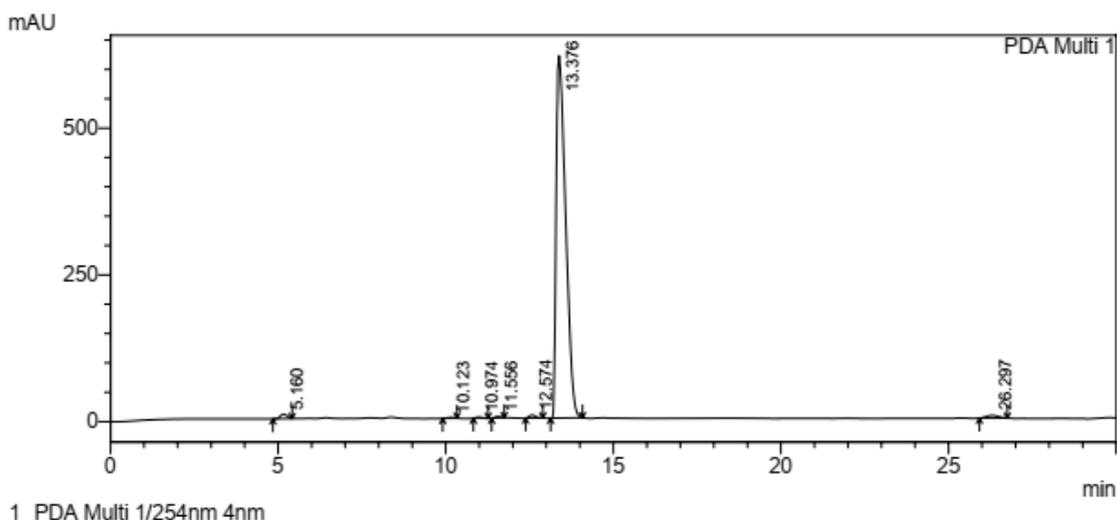
HRMS spectrum of **8h**



¹H NMR spectrum of **9h**



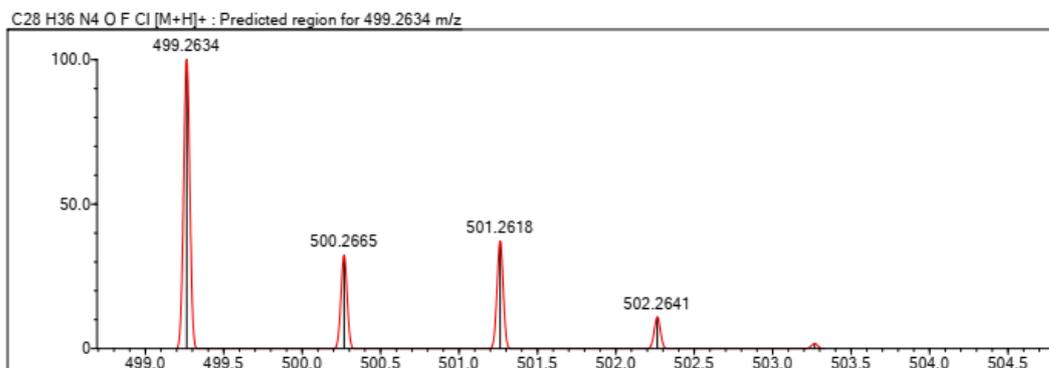
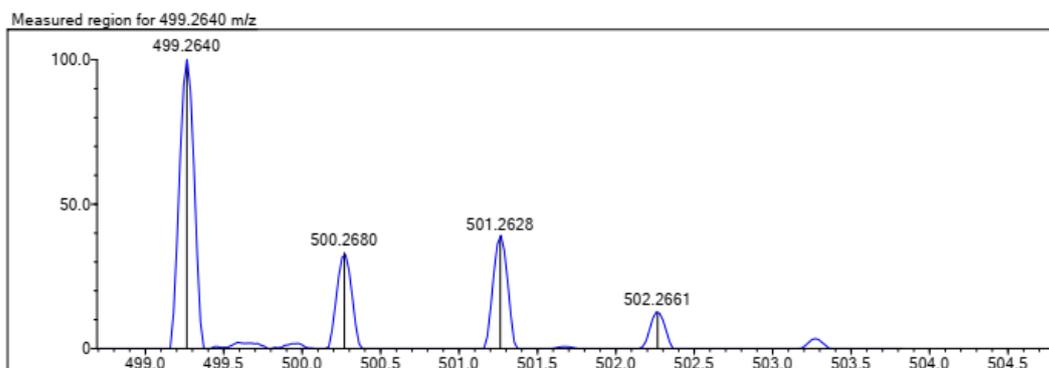
¹³C NMR spectrum of **9h**



PeakTable

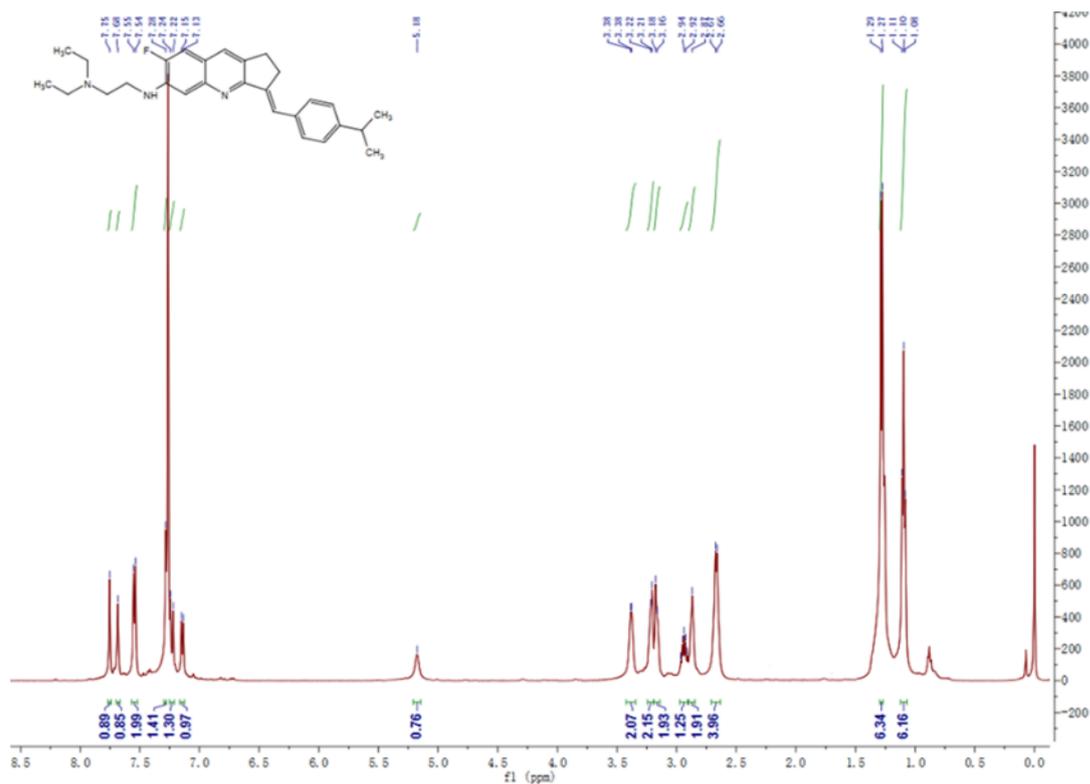
Peak#	Ret. Time	Area	Height	Area %	Height %
1	5.160	112197	7581	0.896	1.184
2	10.123	22671	1520	0.181	0.237
3	10.974	22974	1711	0.184	0.267
4	11.556	28076	2335	0.224	0.365
5	12.574	68015	5164	0.543	0.806
6	13.376	12143237	617418	97.008	96.403
7	26.297	120585	4728	0.963	0.738
Total		12517756	640457	100.000	100.000

HPLC analysis of 9h

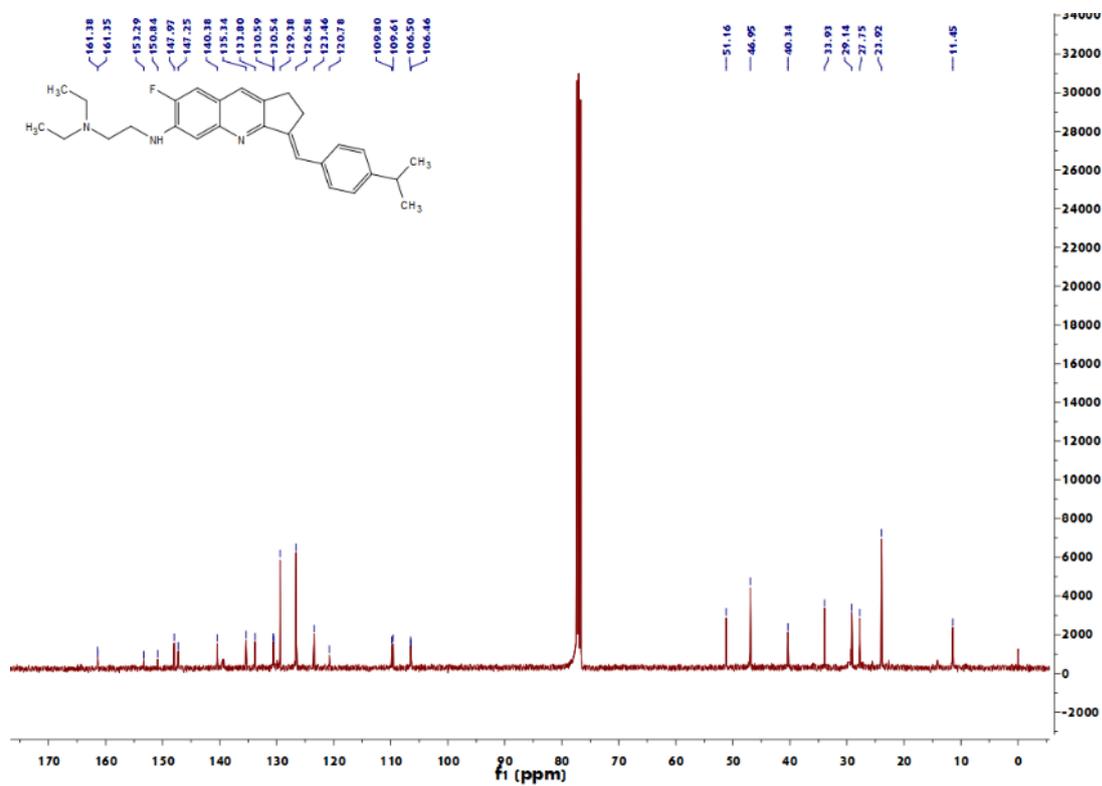


Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	91.12	C28 H36 N4 O F Cl	[M+H] ⁺	499.2640	499.2634	0.6	1.20	91.58	12.0

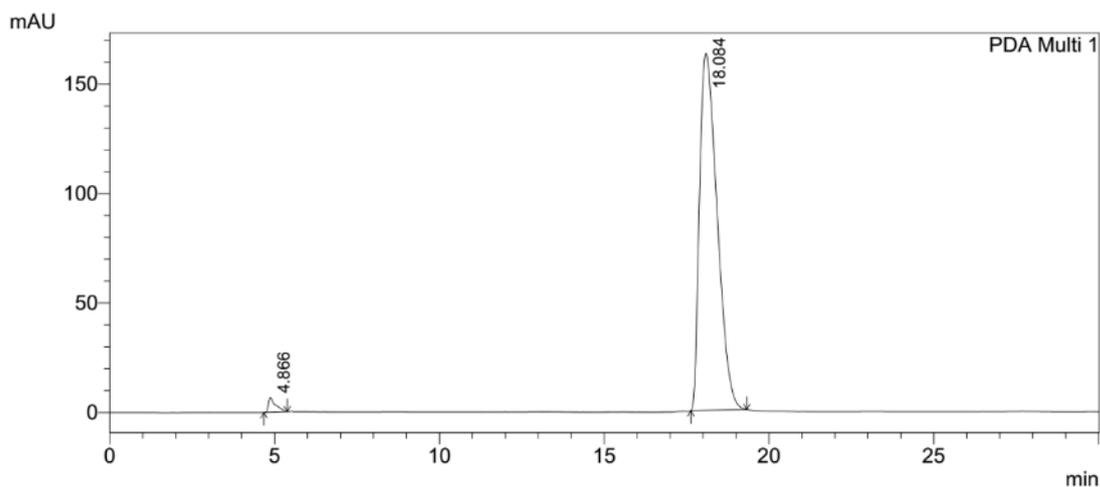
HRMS spectrum of 9h



¹H NMR spectrum of **1i**



¹³C NMR spectrum of **1i**



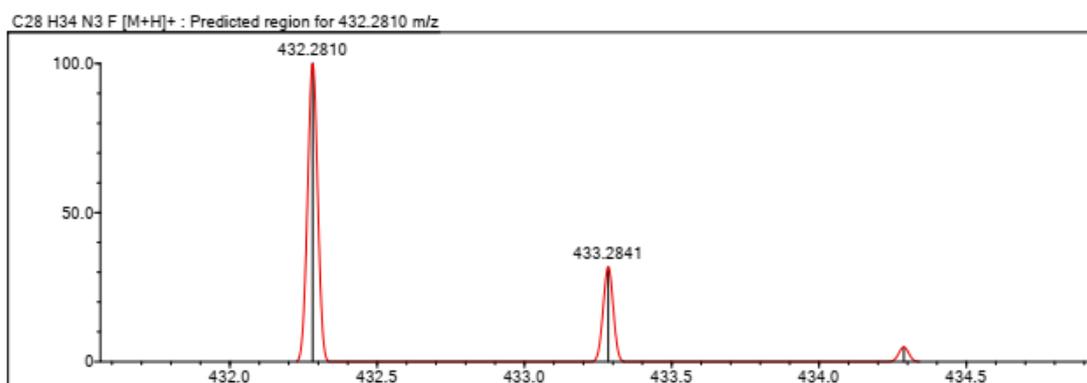
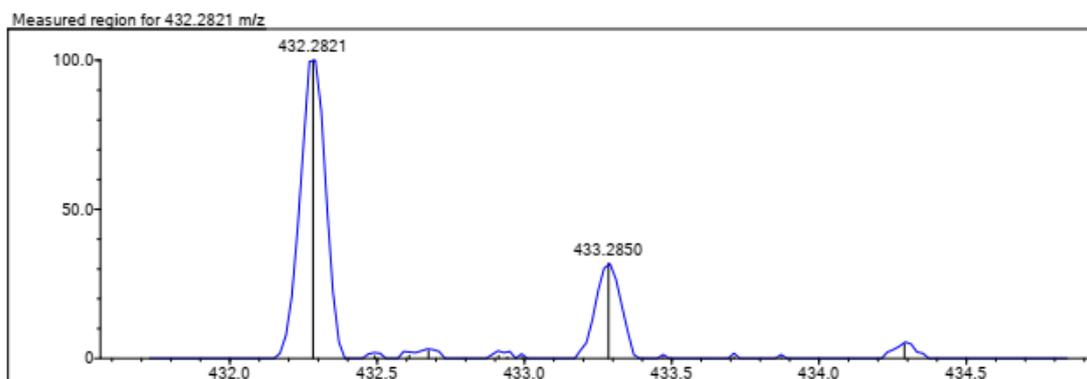
1 PDA Multi 1/254nm 4nm

PeakTable

PDA Ch1 254nm 4nm

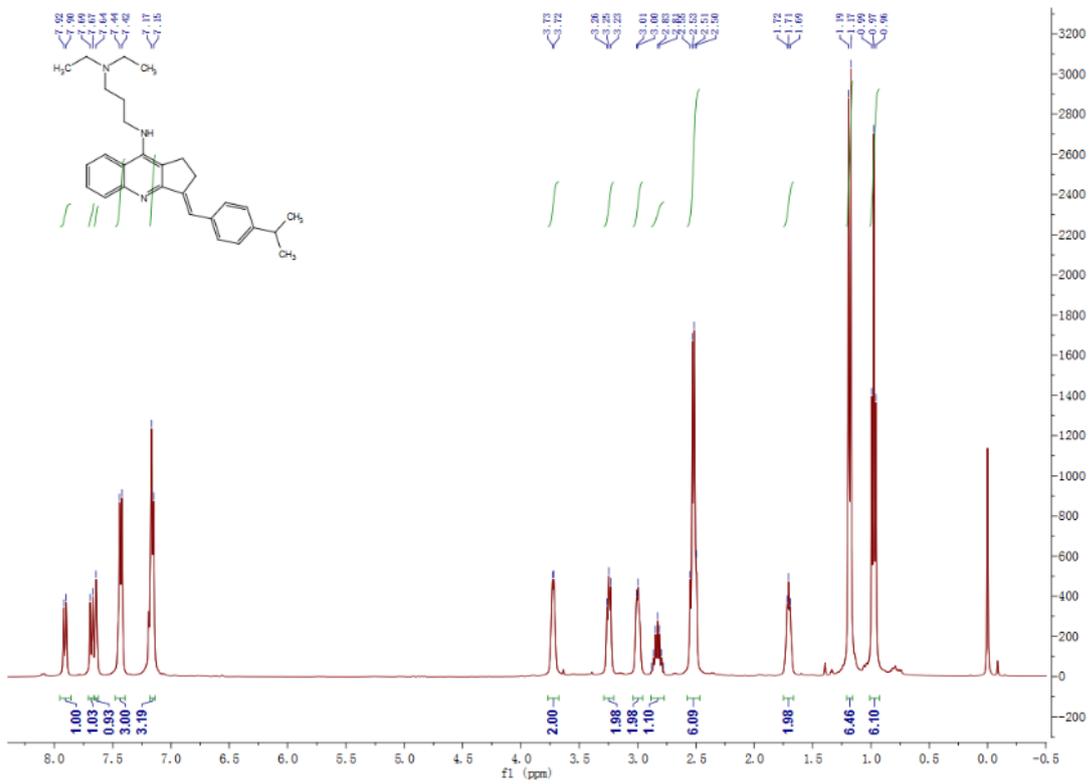
Peak#	Ret. Time	Area	Height	Area %	Height %
1	4.866	101506	6713	1.652	3.952
2	18.084	6041612	163158	98.348	96.048
Total		6143119	169871	100.000	100.000

HPLC analysis of **1i**

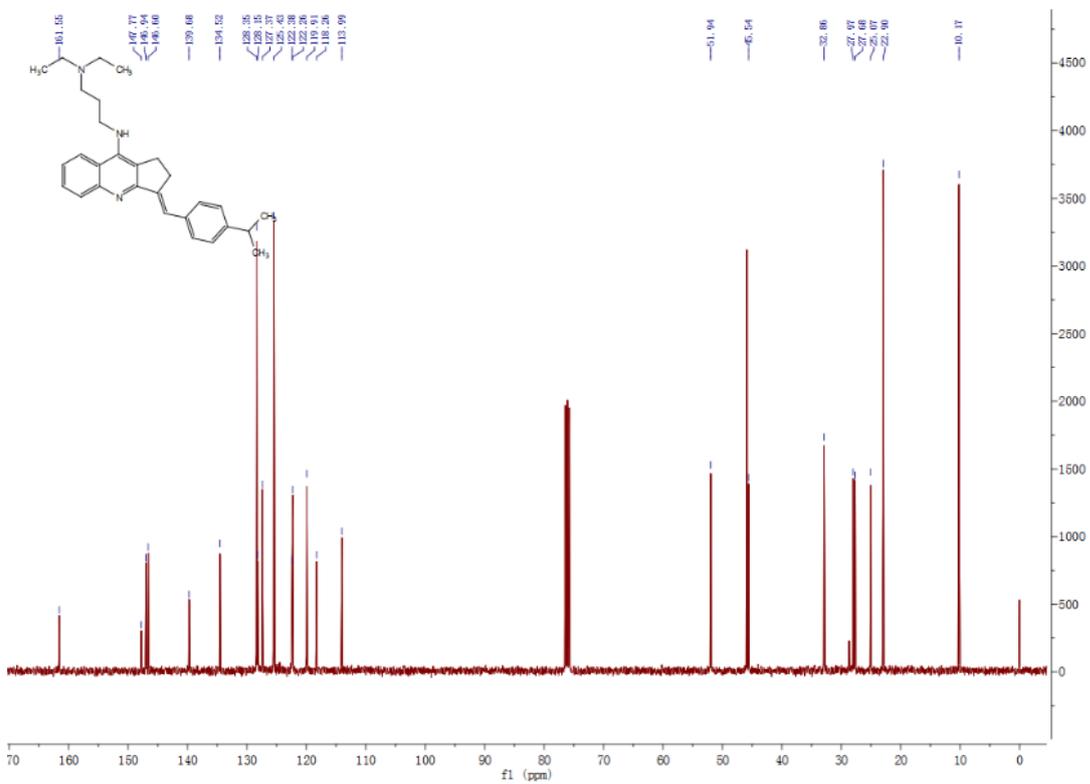


Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
2	79.96	C ₂₈ H ₃₄ N ₃ F	[M+H] ⁺	432.2821	432.2810	1.1	2.54	83.16	13.0

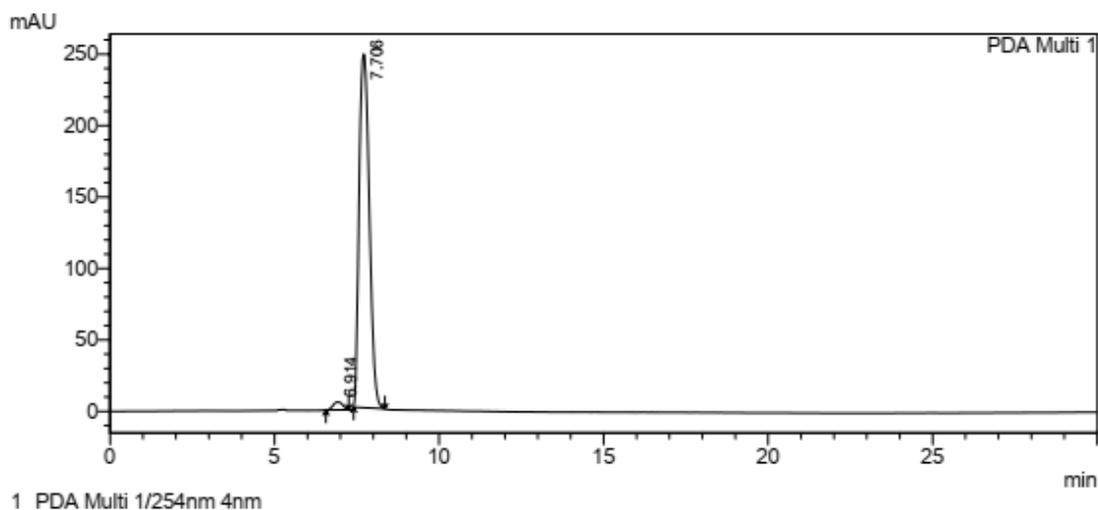
HRMS spectrum of **1i**



¹H NMR spectrum of 1j



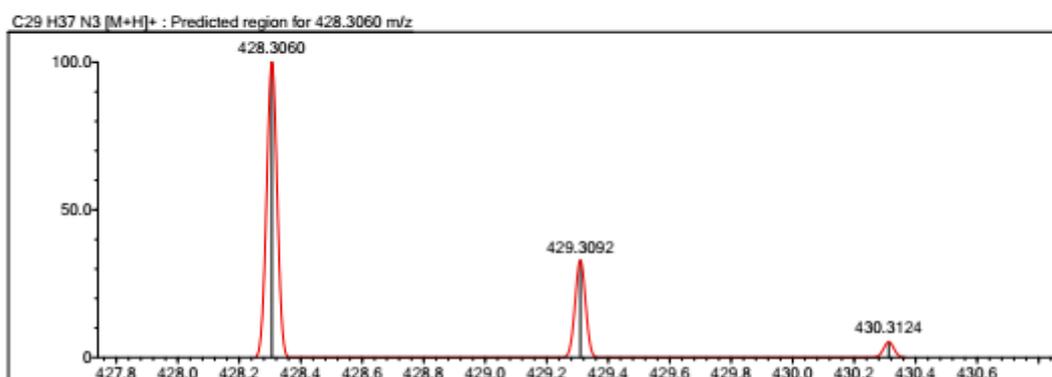
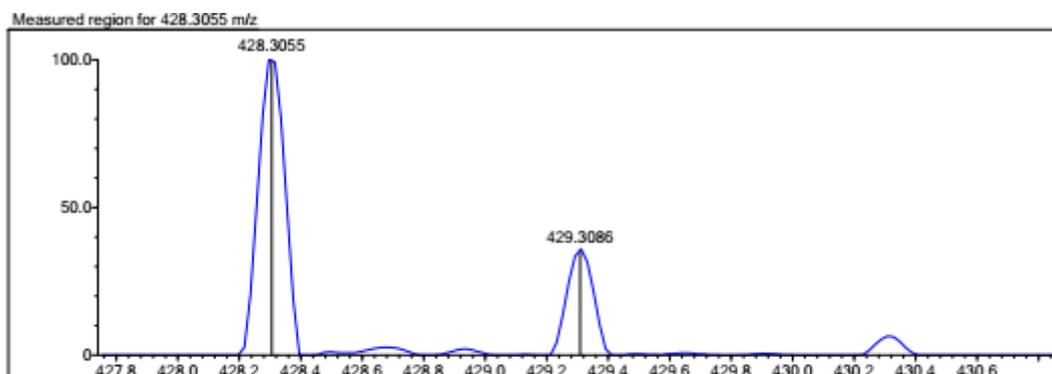
¹³C NMR spectrum of 1j



PeakTable

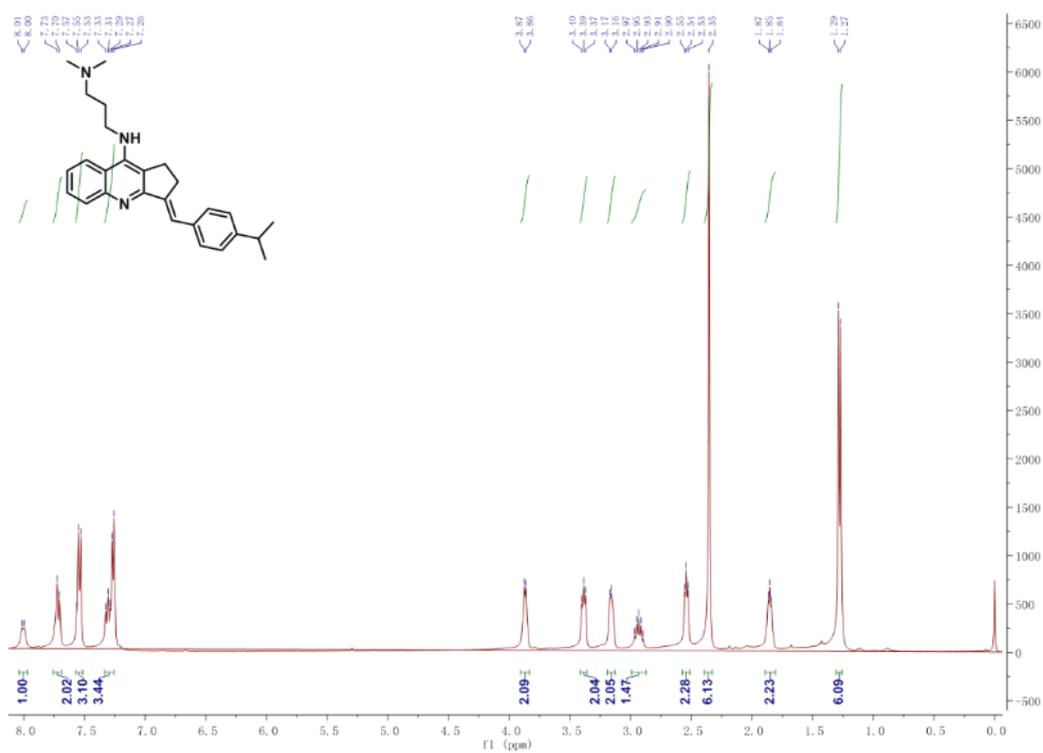
Peak#	Ret. Time	Area	Height	Area %	Height %
1	6.914	113155	5427	2.082	2.145
2	7.706	5322047	247552	97.918	97.855
Total		5435202	252979	100.000	100.000

HPLC analysis of 1j

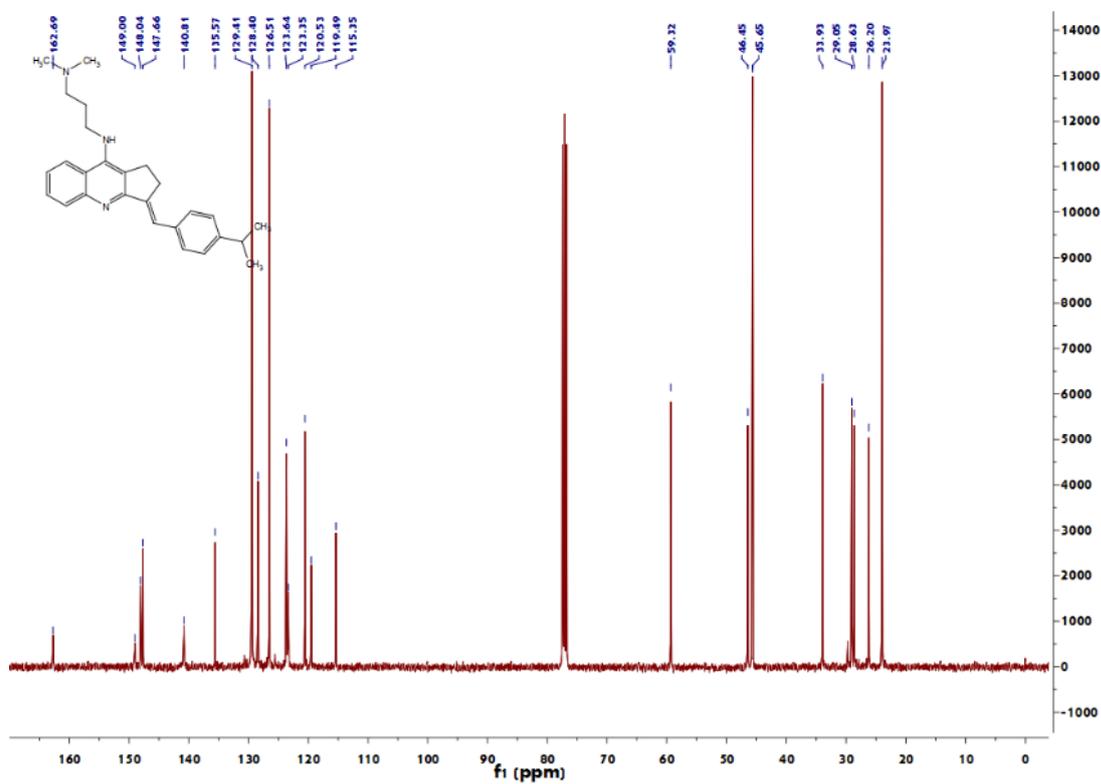


Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	78.40	C29 H37 N3	[M+H] ⁺	428.3055	428.3060	-0.5	-1.17	78.73	13.0

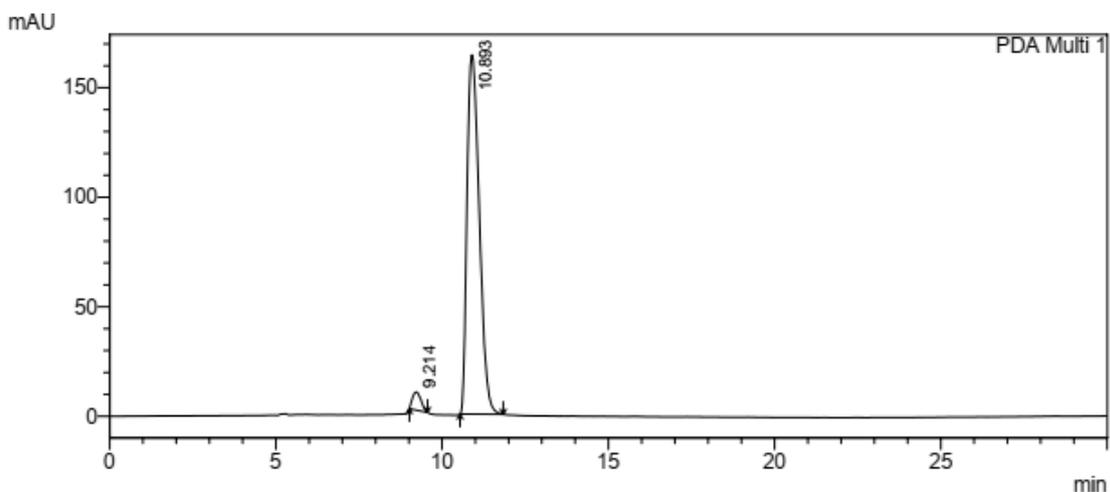
HRMS spectrum of 1j



¹H NMR spectrum of **2j**



¹³C NMR spectrum of **2j**



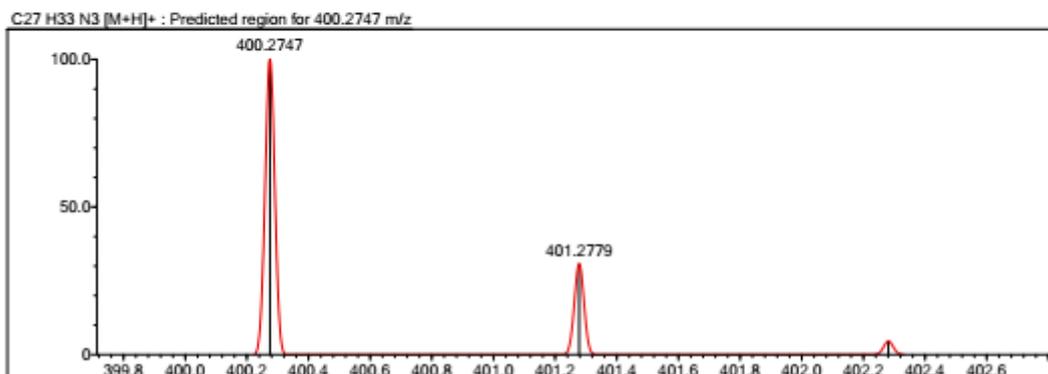
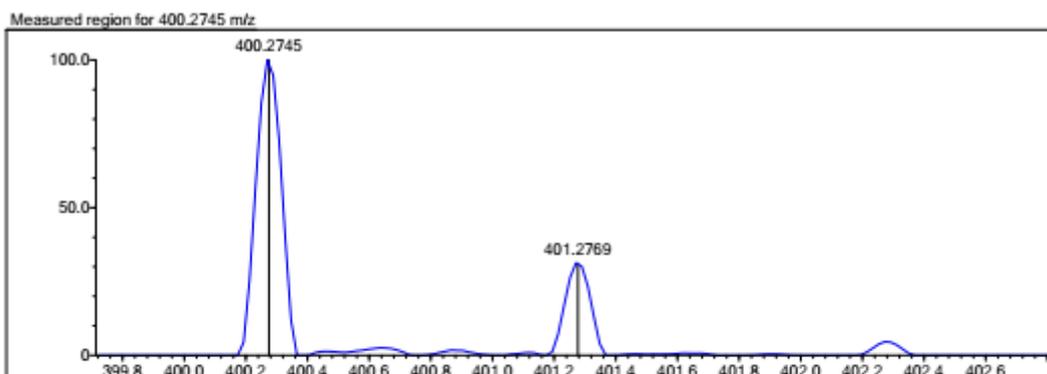
1 PDA Multi 1/254nm 4nm

PeakTable

PDA Ch1 254nm 4nm

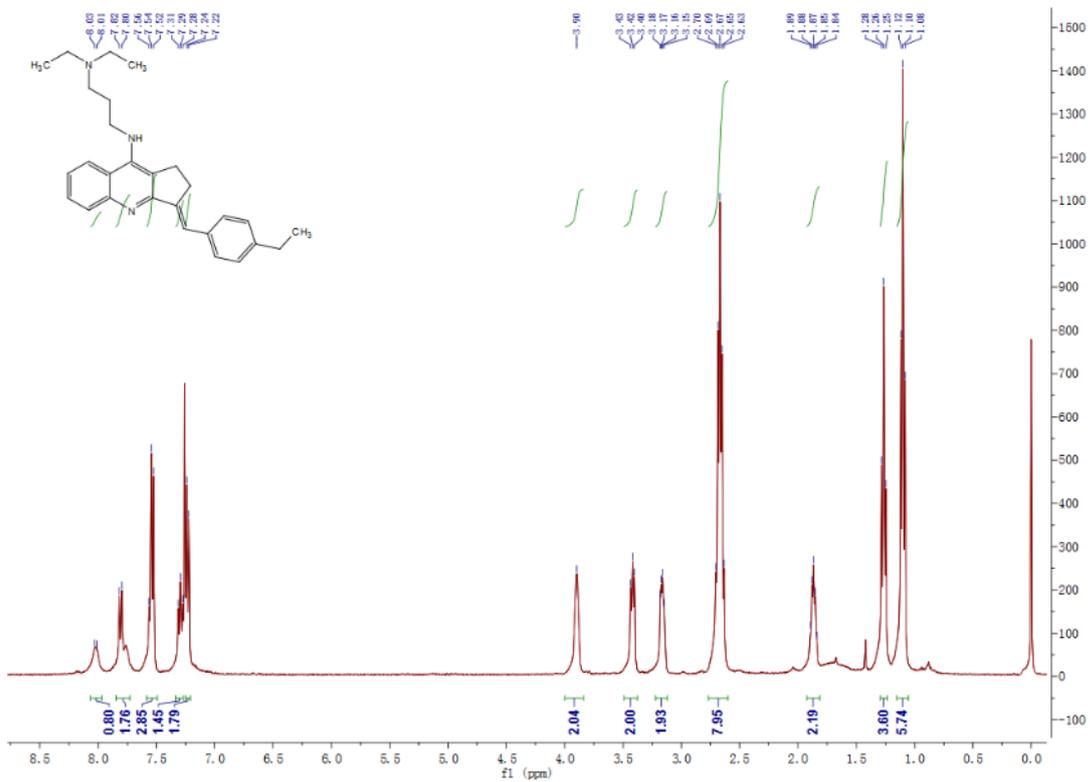
Peak#	Ret. Time	Area	Height	Area %	Height %
1	9.214	147303	8237	3.398	4.781
2	10.893	4188100	164039	96.602	95.219
Total		4335403	172277	100.000	100.000

HPLC analysis of 2j

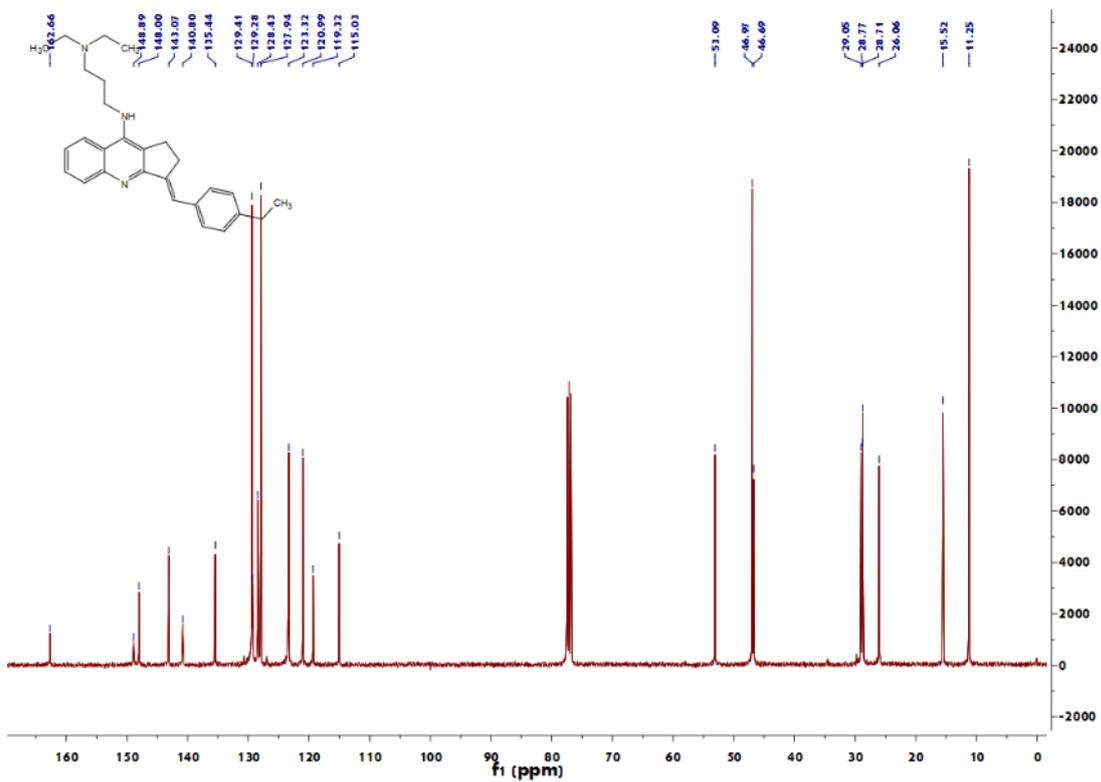


Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	86.29	C27 H33 N3	[M+H] ⁺	400.2745	400.2747	-0.2	-0.50	86.29	13.0

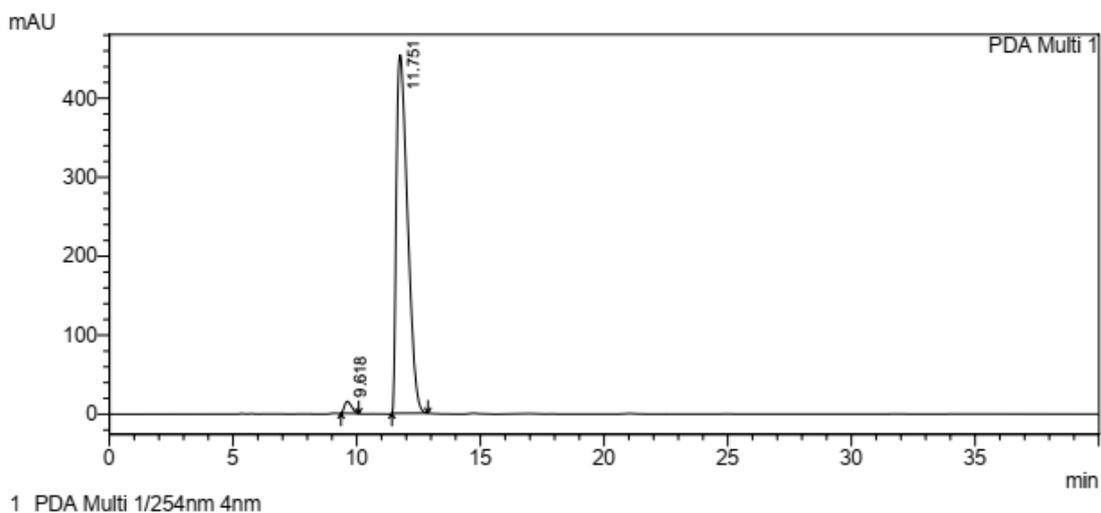
HRMS spectrum of 2j



¹H NMR spectrum of **3j**



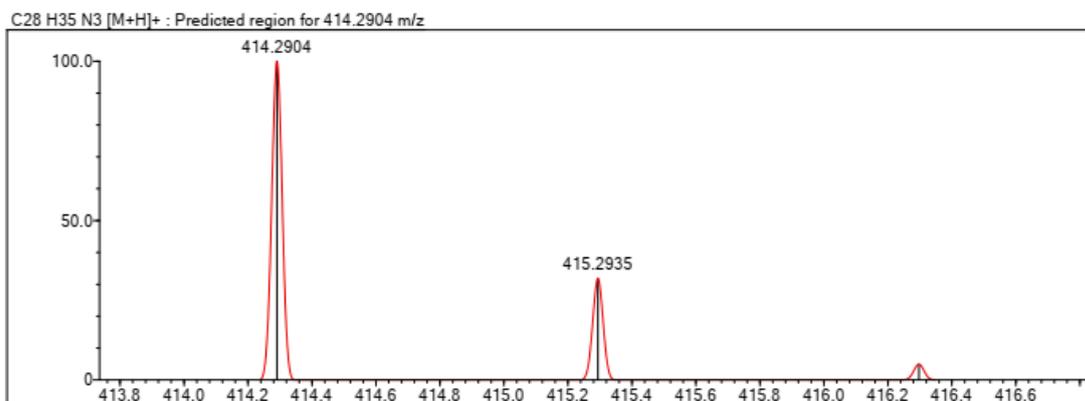
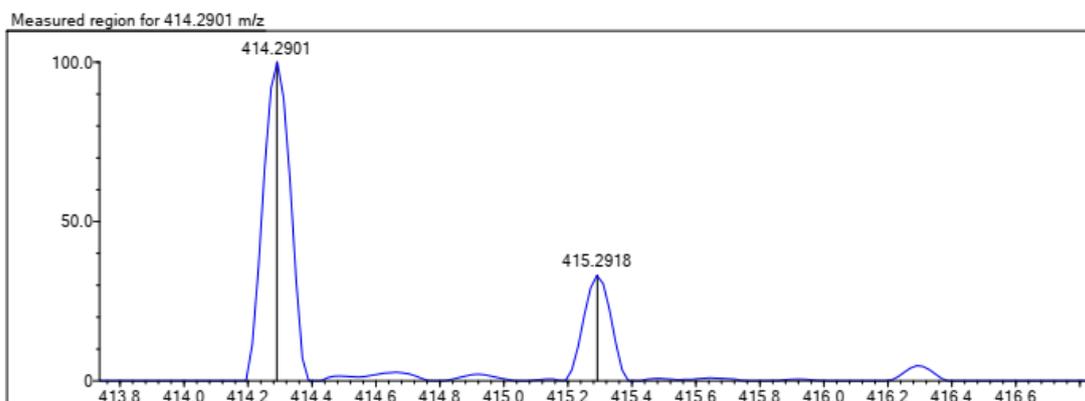
¹³C NMR spectrum of **3j**



PeakTable

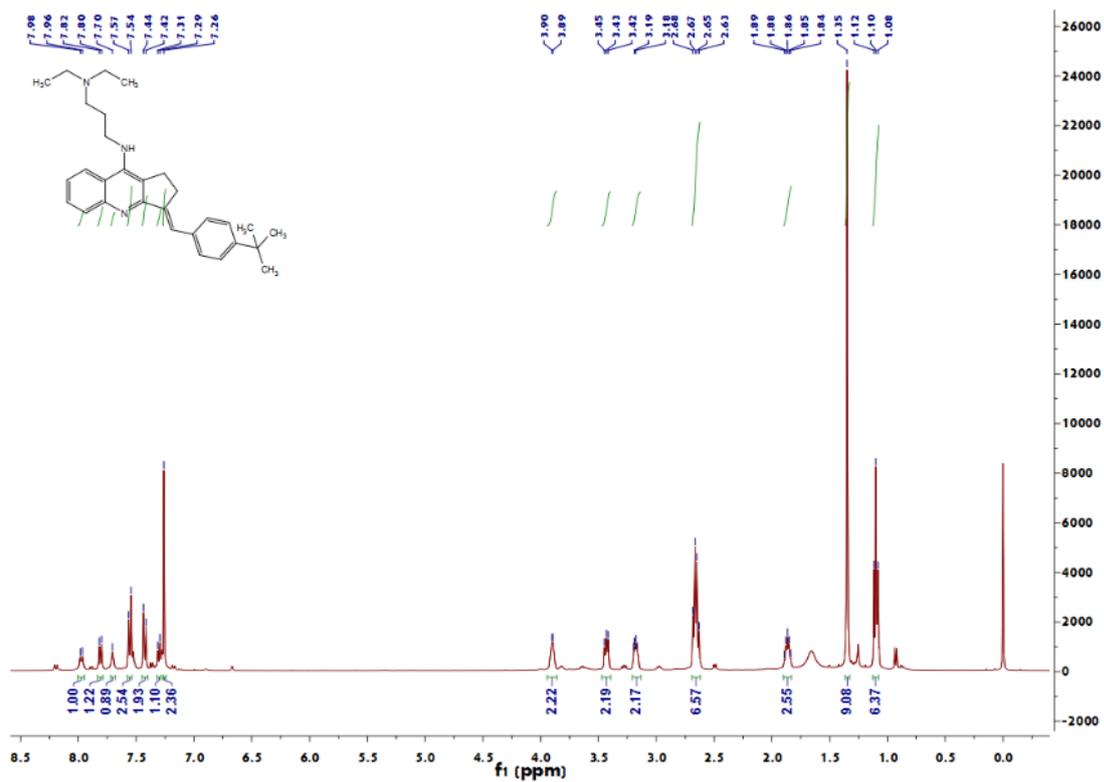
PDA Ch1 254nm 4nm					
Peak#	Ret. Time	Area	Height	Area %	Height %
1	9.618	303061	15013	2.089	3.200
2	11.751	14206240	454158	97.911	96.800
Total		14509301	469171	100.000	100.000

HPLC analysis of 3j

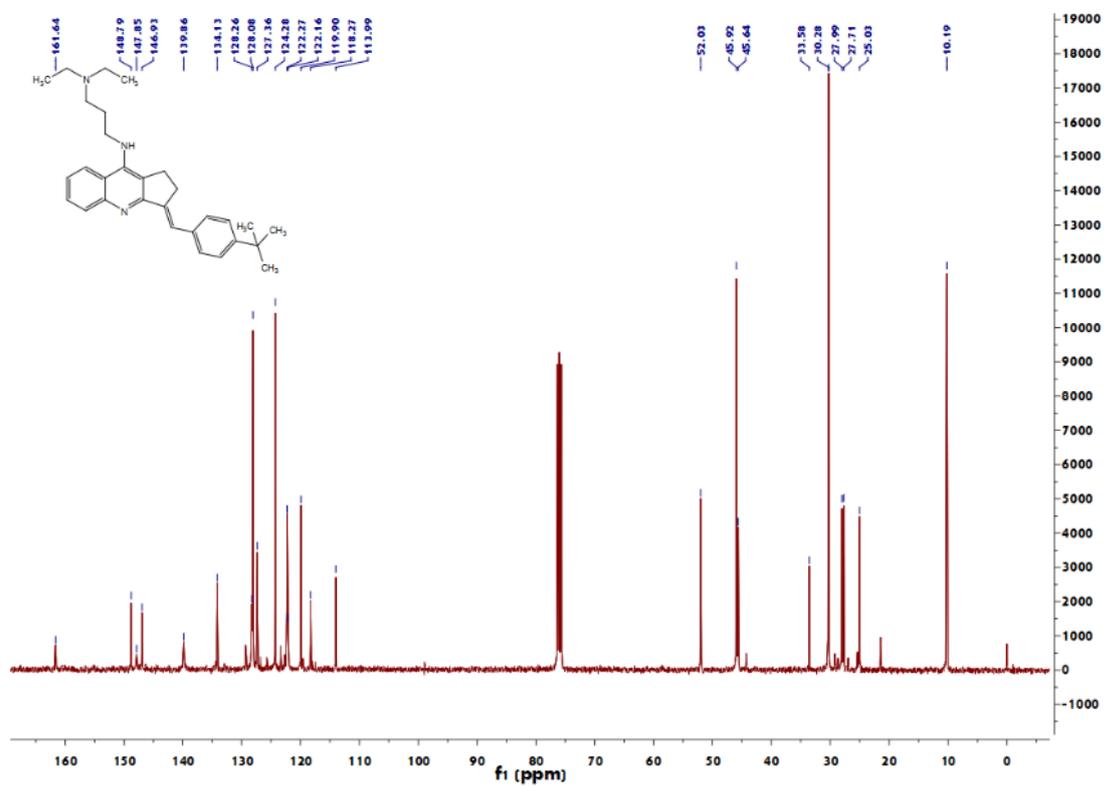


Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
3	78.54	C28 H35 N3	[M+H] ⁺	414.2901	414.2904	-0.3	-0.72	78.54	13.0

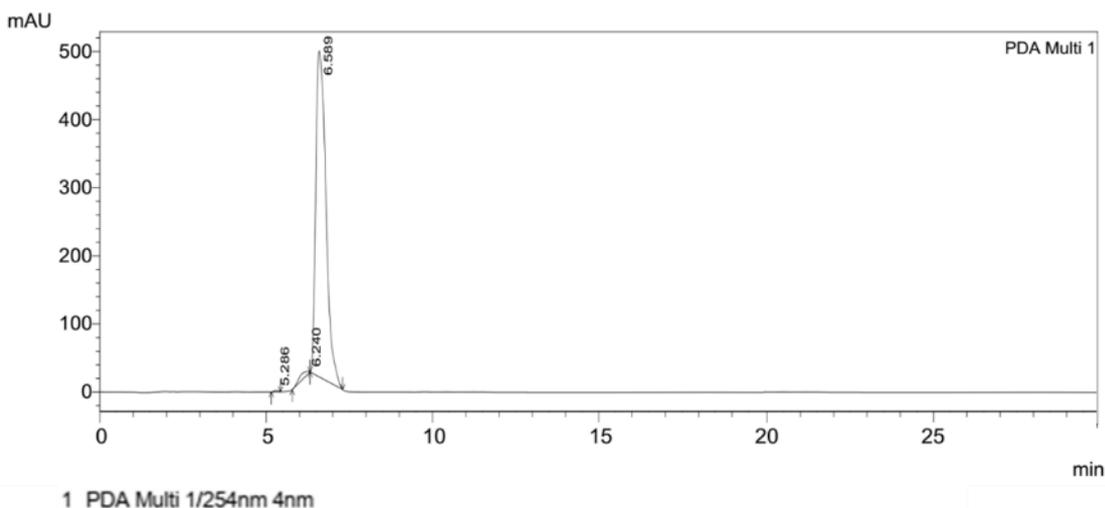
HRMS spectrum of 3j



¹H NMR spectrum of 4j



¹³C NMR spectrum of 4j

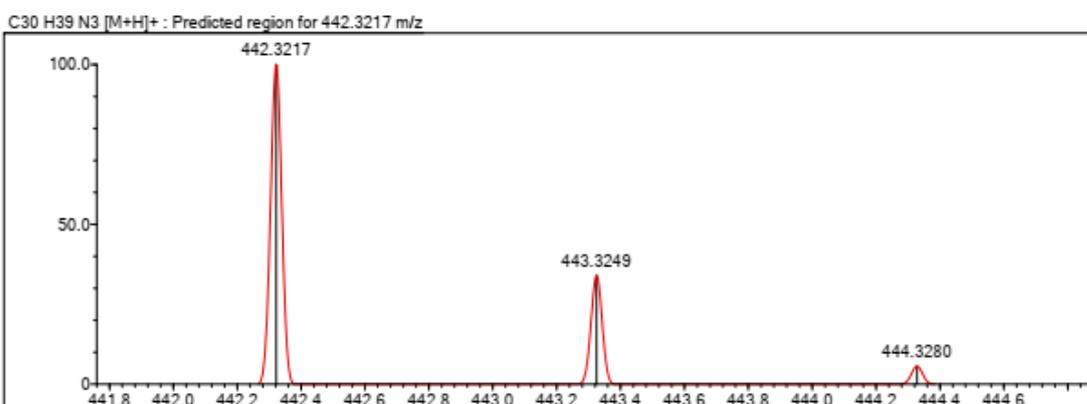
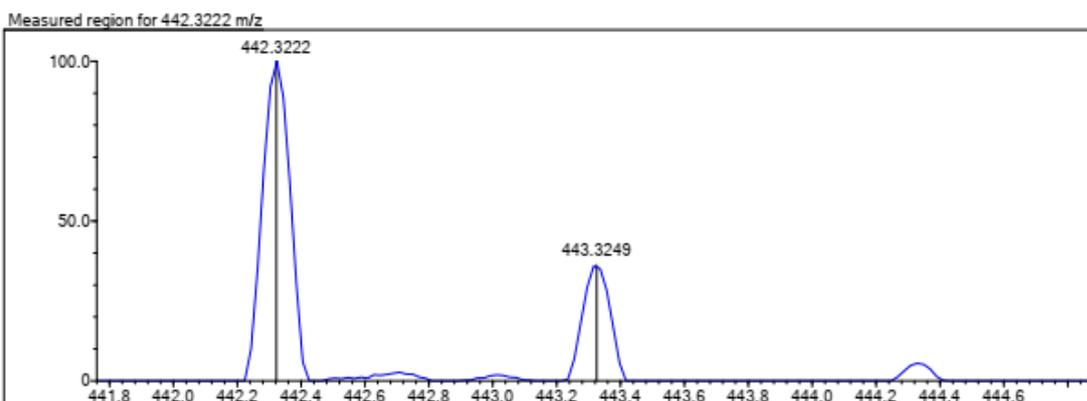


PeakTable

PDA Ch1 254nm 4nm

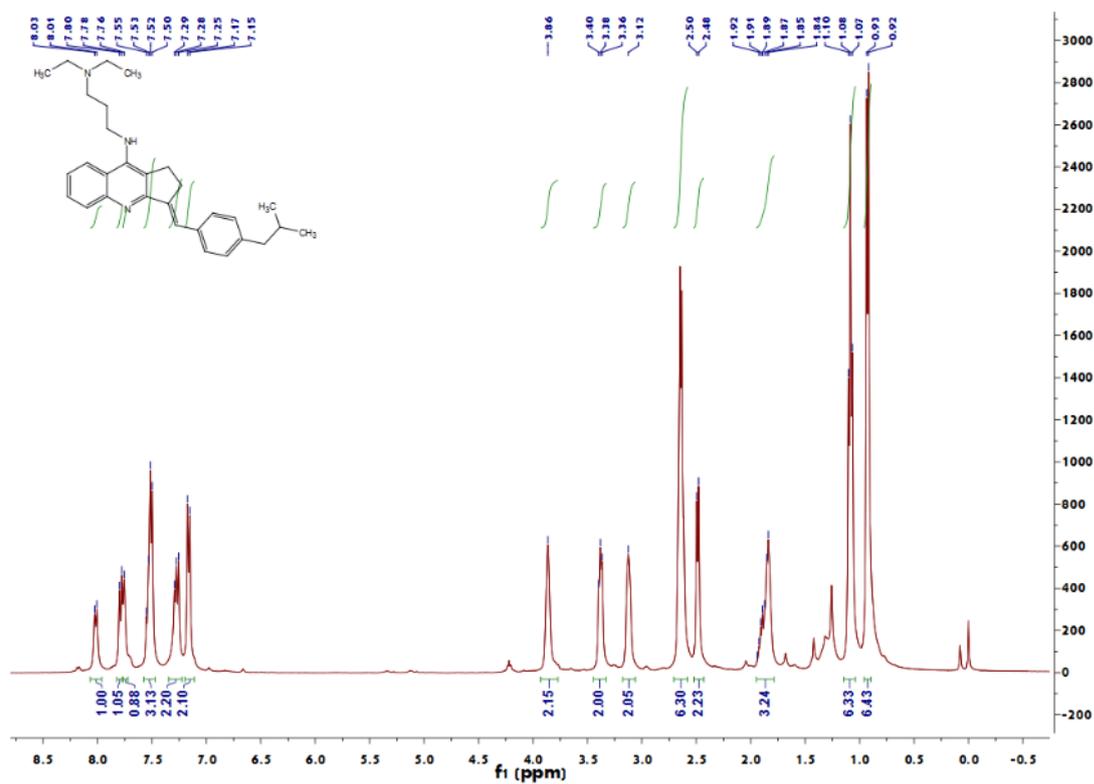
Peak#	Ret. Time	Area	Height	Area %	Height %
1	5.286	18231	2071	0.176	0.426
2	6.240	162483	4888	1.572	1.007
3	6.589	10153883	478701	98.251	98.567
Total		10334597	485660	100.000	100.000

HPLC analysis of 4j

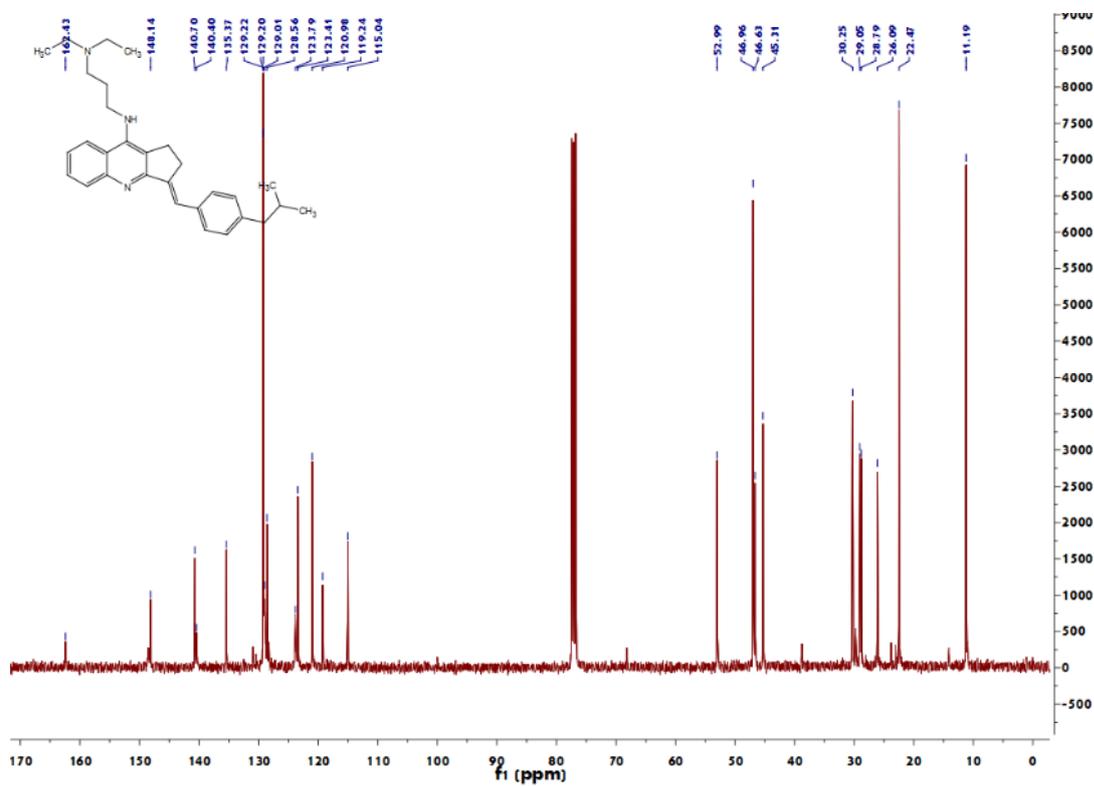


Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Isc	DBE
1	84.58	C30 H39 N3	[M+H] ⁺	442.3222	442.3217	0.5	1.13	84.85	13.0

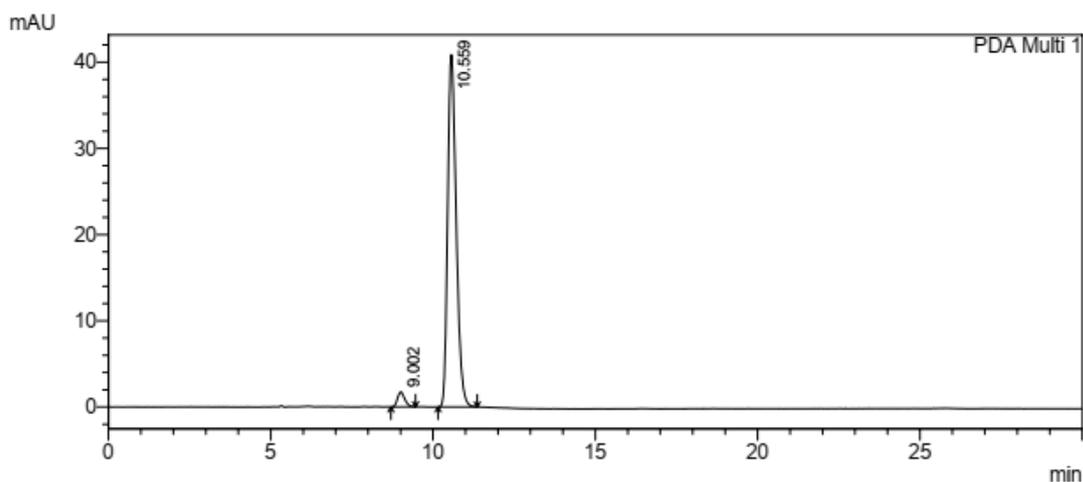
HRMS spectrum of 4j



¹H NMR spectrum of 5j



¹³C NMR spectrum of 5j



1 PDA Multi 1/254nm 4nm

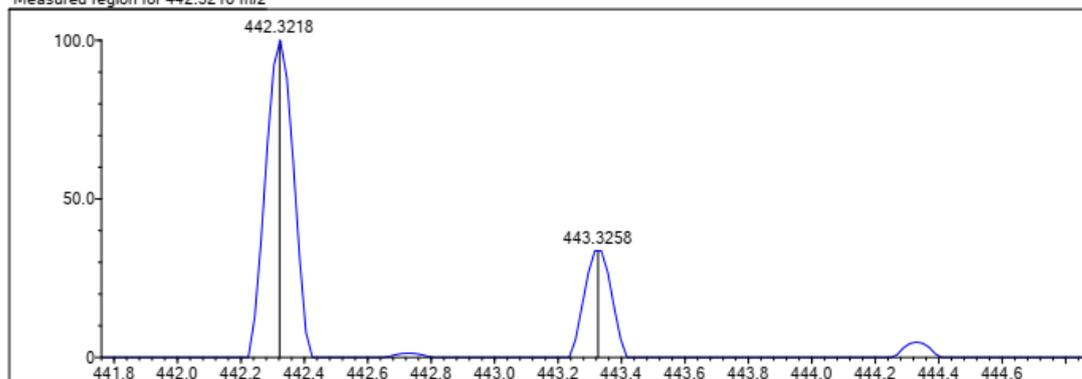
PeakTable

PDA Ch1 254nm 4nm

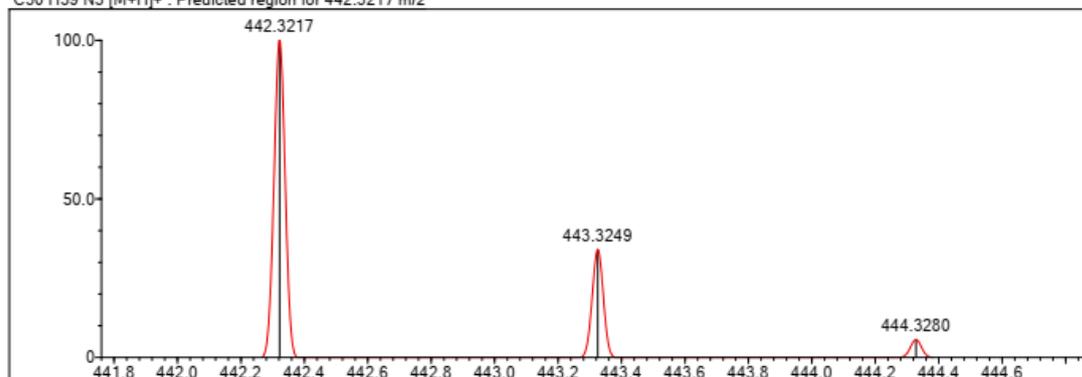
Peak#	Ret. Time	Area	Height	Area %	Height %
1	9.002	26683	1737	3.441	4.076
2	10.559	748800	40872	96.559	95.924
Total		775483	42608	100.000	100.000

HPLC analysis of 5j

Measured region for 442.3218 m/z



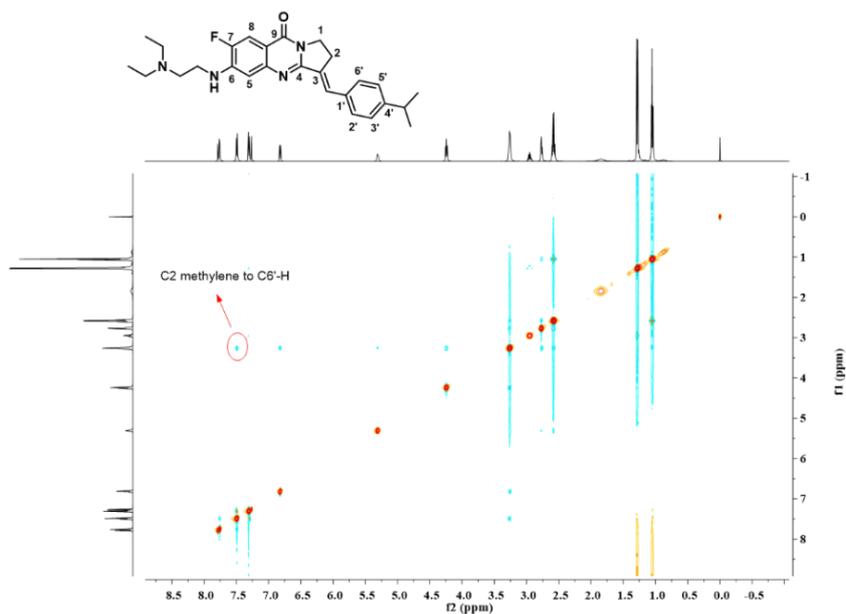
C30 H39 N3 [M+H]⁺ : Predicted region for 442.3217 m/z



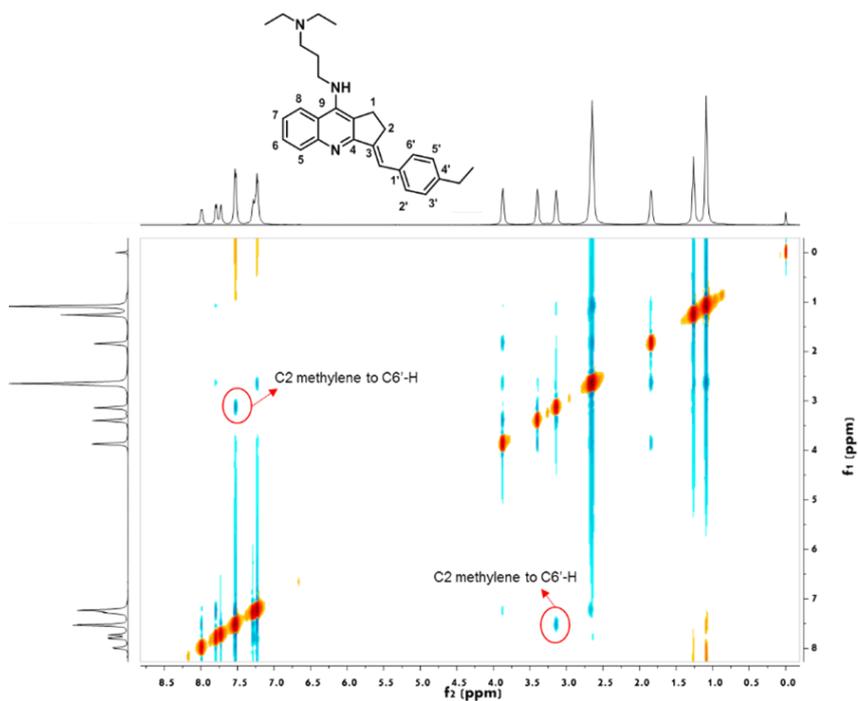
Rank	Score	Formula (M)	Ion	Meas. m/z	Pred. m/z	Df. (mDa)	Df. (ppm)	Iso	DBE
1	86.78	C30 H39 N3	[M+H] ⁺	442.3218	442.3217	0.1	0.23	86.78	13.0

HRMS spectrum of 5j

NOESY Spectrum and Polarimetric Analyses



NOESY spectrum of compound **3f**



NOESY spectrum of compound **3j**

Polarimetric analyses of compound **1g** and **3g**

Compound	1g	3g	D-glucose
Specific optical rotation [α] (deg·mL)/(g·mm)	0.00	0.00	52.03