Supporting information

Synthesis and pharmacological characterization of new photo-caged agonist for histamine H_3 and H_4 receptors

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[#] Both authors contributed equally.

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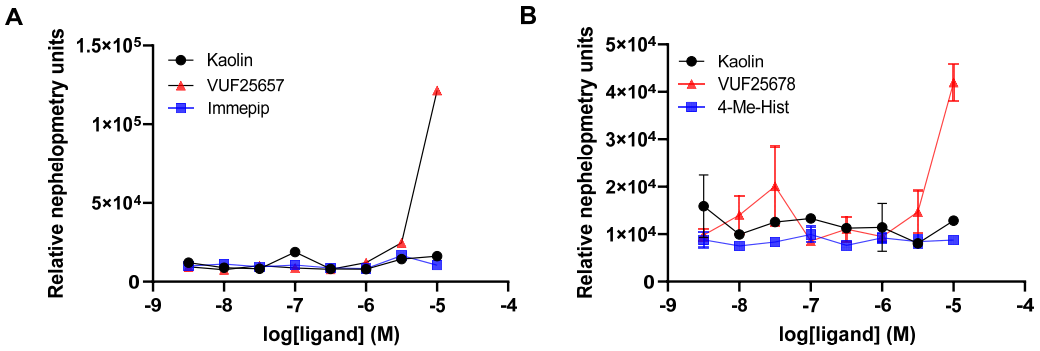

Figure S1. Representative plots for nephelometry measurements at different concentrations of parent compounds (immepip and 4-methylhistamine) and caged-compounds VUF25657 and VUF25678 in the dark. A kaolin dispersion was used as a positive control. Experiments were performed in duplicate in 50 mM Tris-HCl buffer (pH 7.4)/1 % DMSO.

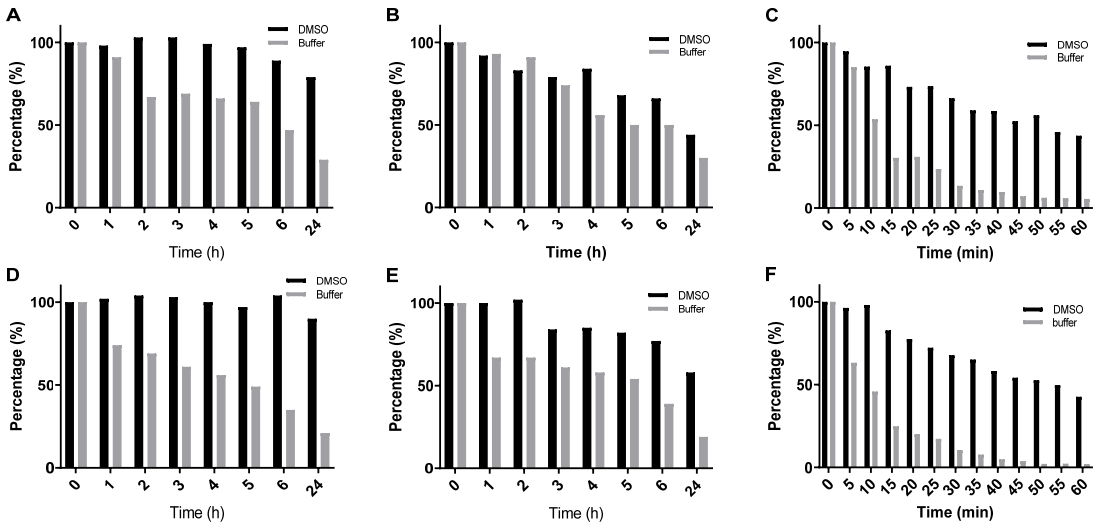

Figure S2. Chemical stability of VUF25657 (A/B/C) and VUF25678 (D/E/F) under dark, red light and ambient light. VUF25657 or VUF25678 (3.2 µM) was incubated in Tris-HCl buffer (50 mM, pH 7.4)/1% DMSO at room temperature and analysis by LC-MS (λ = 254 nm, area % of BODIPY-caged compounds versus internal standard (1,3,5-trimethoxylbenzene) was performed at different time points. Black columns: VUF25657 or VUF25678 in DMSO; Grey columns: VUF25657 or VUF25678 in Tris-HCl buffer (50 mM, pH 7.4)/1% DMSO.

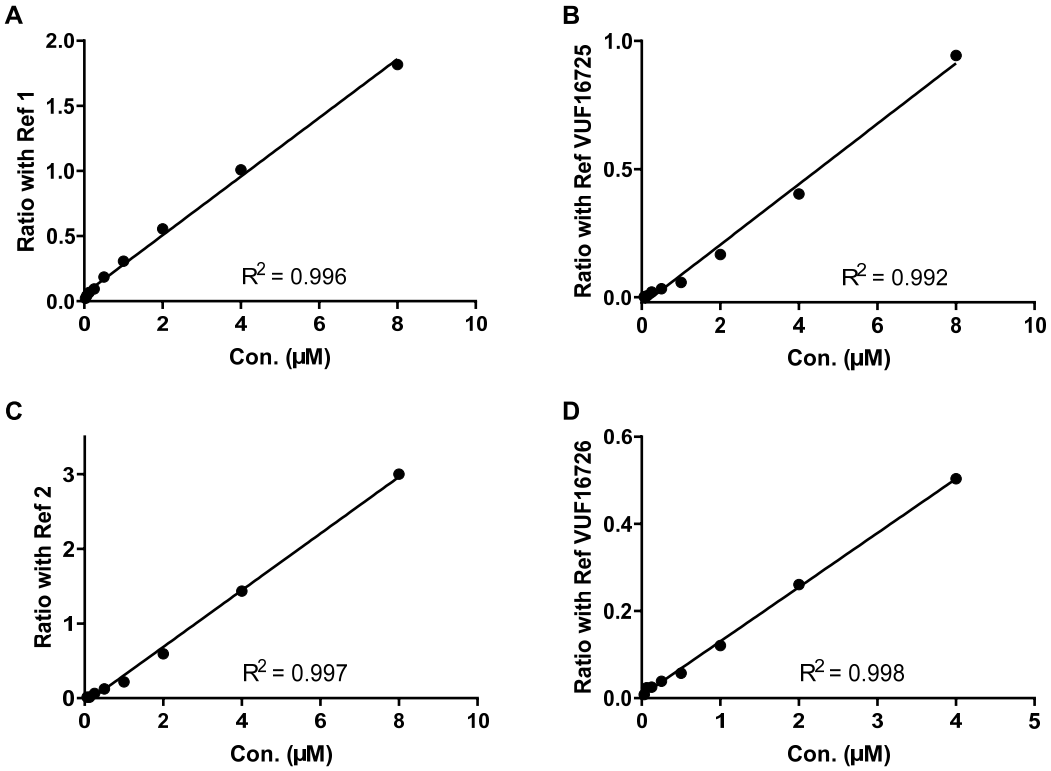

Figure S3. MS calibration curves of immepip, 4-methylhistamine and BODIPY-caged compounds VUF25657 and VUF25678 with their corresponding reference compounds. The ratio of peak areas is plotted. A, calibration curve of immepip with 3-dimethylaminobenzoic acid from Sigma-Aldrich (CAS: 99-64-9); B, calibration curve of VUF25657 with VUF16725 (from our in-house library); C, calibration curve of 4-methylhistamine with (4-fluorophenyl)methanamine Sigma-Aldrich (CAS: 140-75-0); D, calibration curve of VUF25658 with VUF16726 (from our in-house library).

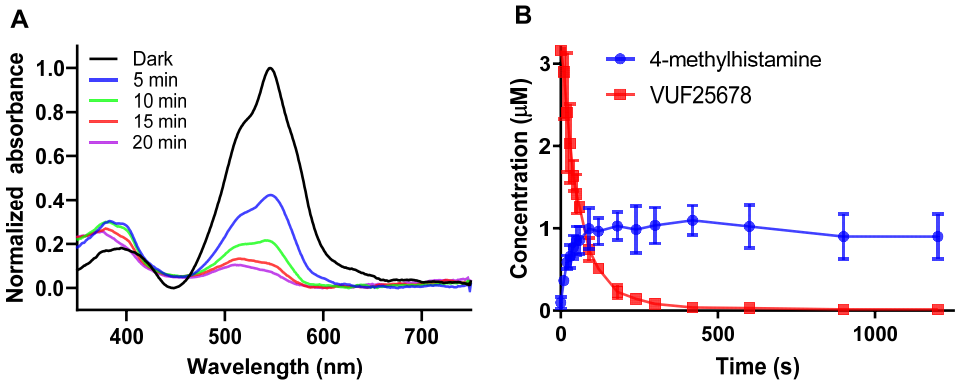

Figure S4. Photo-uncaging followed by UV-Vis and LC-MS analysis. (A) VUF25678 (3.2 µM) was illuminated under 560 nm in Tris-HCl buffer (50 mM, pH 7.4)/1% DMSO at room temperature and a UV-Vis spectrum measured at intervals; (B) VUF25678 (3.2 µM) was illuminated under green LED light in Tris-HCl buffer (50 mM, pH 7.4)/1% DMSO at 37 ℃ and LC-MS analysis performed at different time points.

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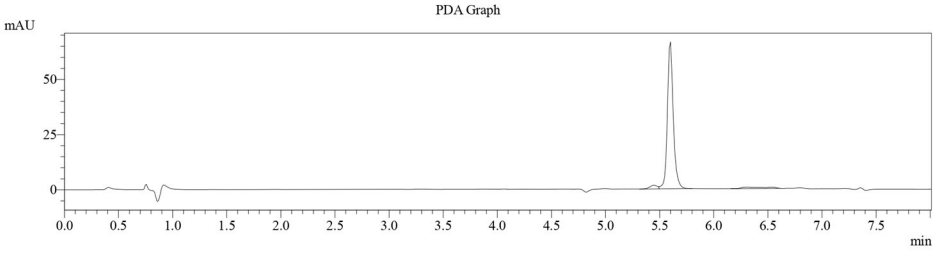

PeakTable

PDA Ch1 254nm 4nm

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2		5.592	249100	93.042
3		6.299	10075	3.763

MS Chromatogram

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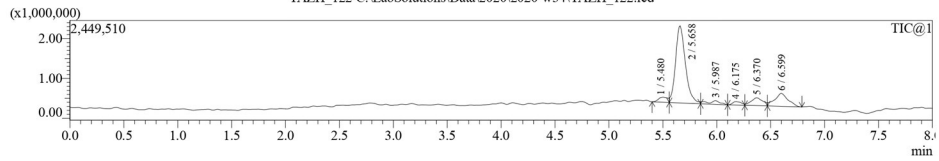

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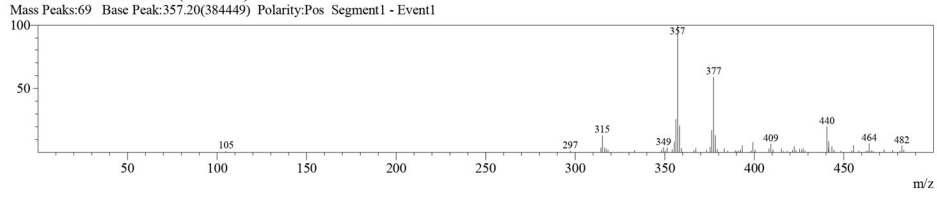

MS Spectrum Table

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Mass Peaks:69 Base Peak:357.20(384449) Polarity:Pos Segment1 - Event1

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1	104.95	4498	1.17				10	349.20	15142	3.94			
2	297.25	4923	1.28				11	350.25	5921	1.54			
3	314.30	14792	3.85				12	351.30	14934	3.88			
4	315.20	51583	13.42				13	354.15	9459	2.46			
5	316.25	15112	3.93				14	355.30	32639	8.49			
6	317.25	11344	2.95				15	356.20	99960	26.00			
7	318.30	8087	2.10				16	357.20	384449	100.00			
8	333.15	7317	1.90				17	358.25	80507	20.94			
9	348.15	7420	1.93				18	359.35	12656	3.29			
19	366.30	5649	1.47				45	423.30	6657	1.73			
20	367.30	12989	3.38				46	425.30	11057	2.88			
21	373.35	7694	2.00				47	426.30	8104	2.11			
22	375.30	16803	4.37				48	427.25	12709	3.31			
23	376.20	66869	17.39				49	428.30	5596	1.46			
24	377.25	226563	58.93				50	440.50	77599	20.18			
25	378.25	52045	13.54				51	441.50	13354	3.47			

Figure S5. LCMS chromatogram of intermediate **2**.

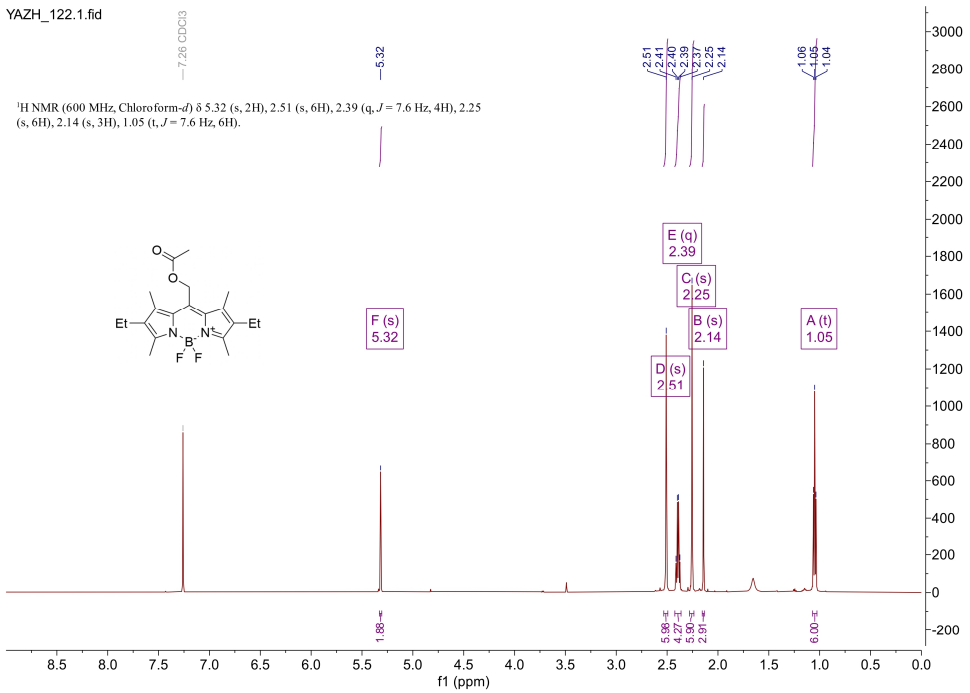

Figure S6. ^{1}H NMR spectrum of intermediate **2**.

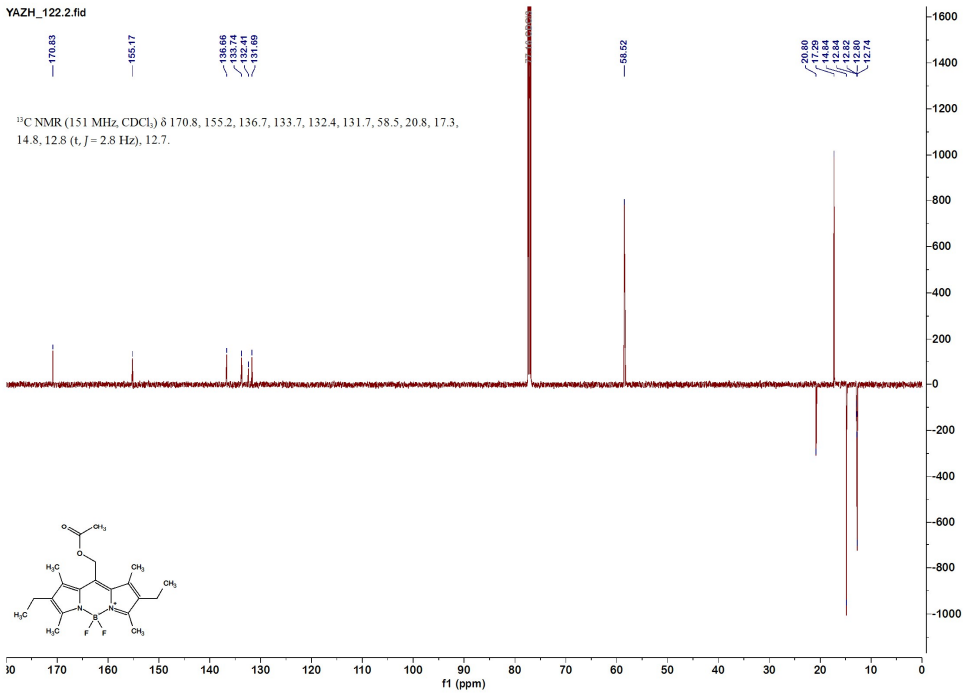

Figure S7. ^{13}C NMR spectrum of intermediate **2**.

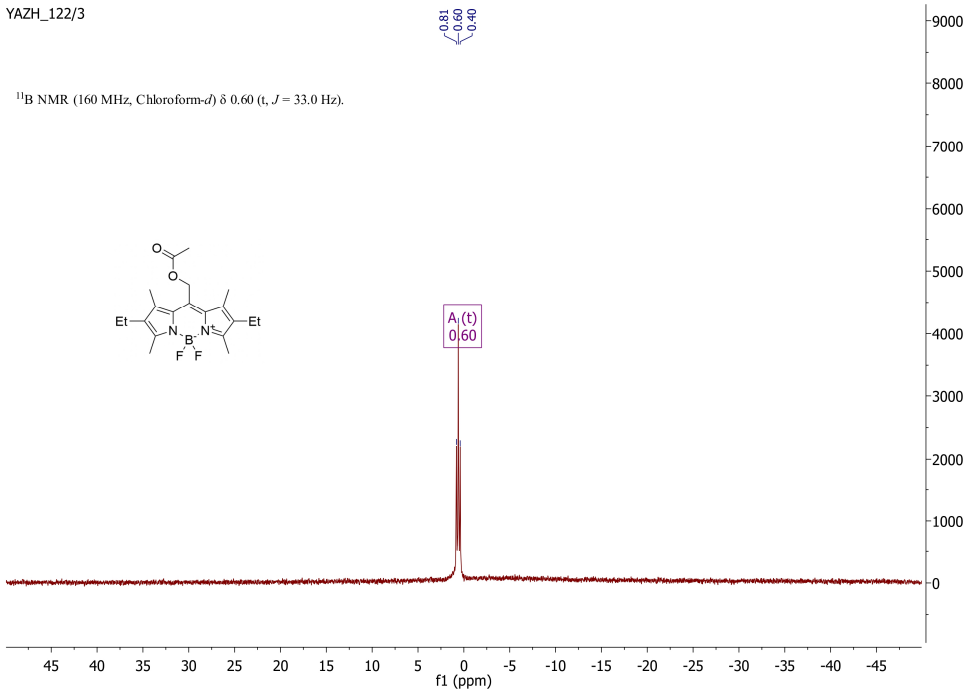

Figure S8. ^{11}B NMR spectrum of intermediate **2**.

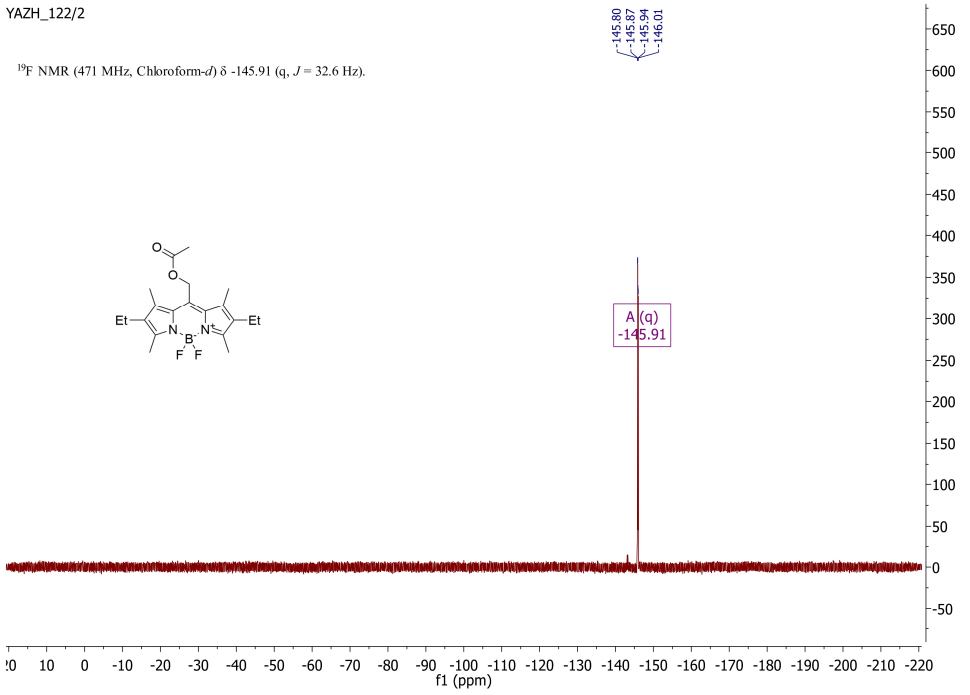

Figure S9. ^{19}F NMR spectrum of intermediate **2**.

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Background File	: azo blanco 24082020.lcd
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Report Format	: DefaultLCMS.lcr
Tuning File	: C:\LabSolutions\Tuning\Tuning-ESI-pos-neg01072015a.lct
Processed by	: Admin
Modified Date	: 24/8/2020 10:31:04 AM

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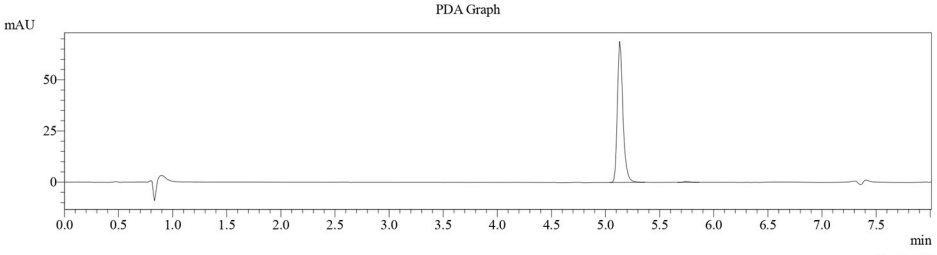


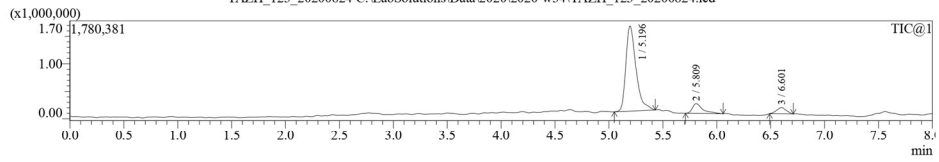
PeakTable

PDA Ch1 254nm 4nm

Peak#	Name	Ret. Time	Area	Area %
1		5.127	247298	99.395
2		5.739	1504	0.605

MS Chromatogram

YAZH_123_20200824 C:\LabSolutions\Data\2020\2020-w34\YAZH_123_20200824.lcd



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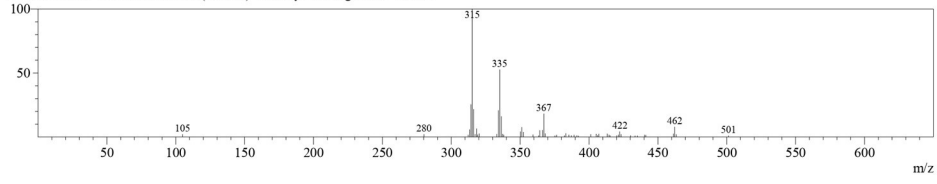

MS Spectrum Table

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1	105.00	8069	2.19				9	318.35	24544	6.66			
2	280.25	8193	2.22				10	319.15	6928	1.88			
3	312.30	6651	1.80				11	320.25	10001	2.71			
4	313.30	21923	5.95				12	333.10	8590	2.33			
5	314.30	94237	25.57				13	334.30	76925	20.88			
6	315.20	368490	100.00				14	335.20	194192	52.70			
7	316.20	79927	21.69				15	336.30	59729	16.21			
8	317.25	8518	2.31				16	337.35	8941	2.43			

Figure S10. LCMS chromatogram of intermediate **3**.

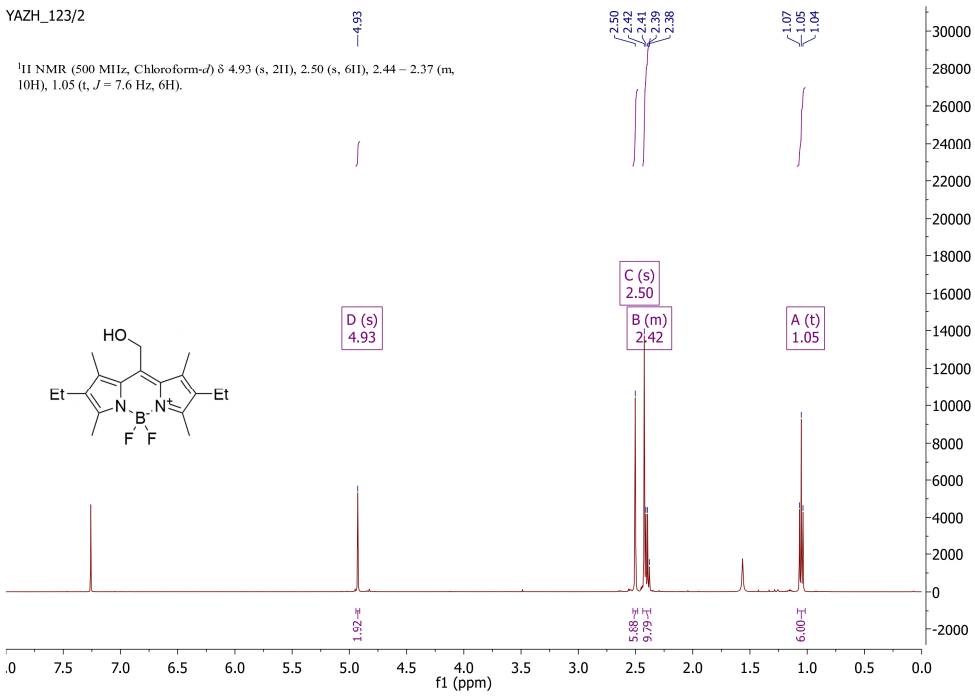

Figure S11. ^{1}H NMR spectrum of intermediate **3**.

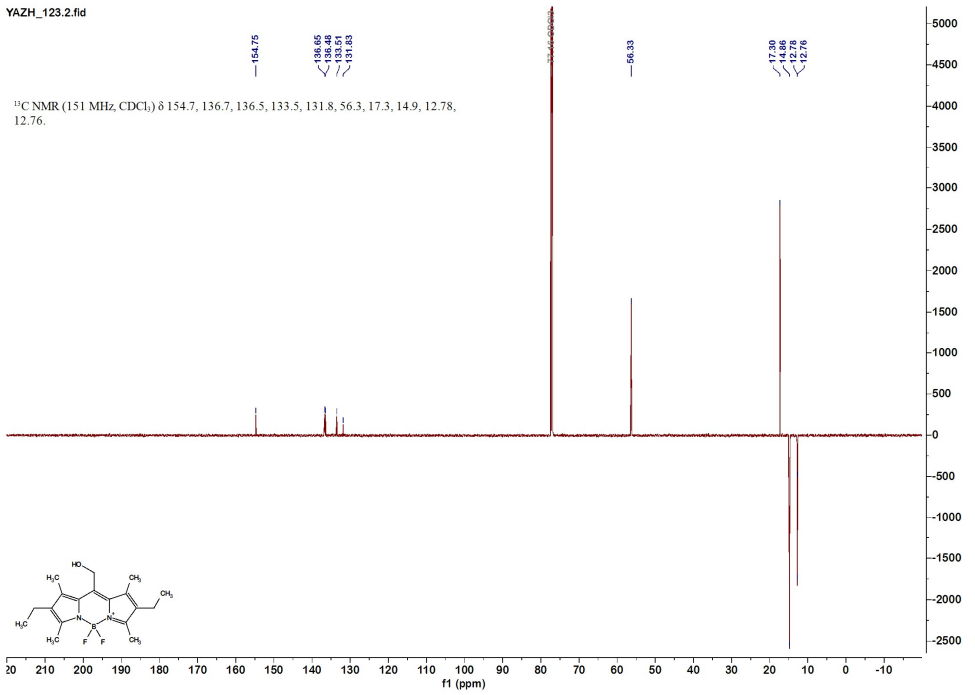

Figure S12. ^{13}C NMR spectrum of intermediate **3**.

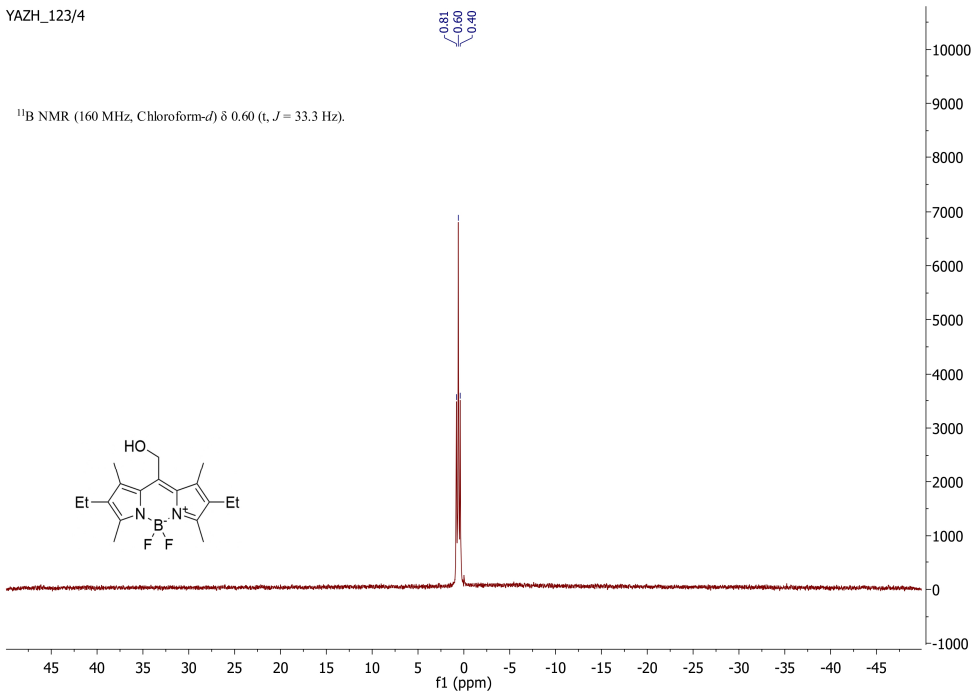

Figure S13. ^{11}B NMR spectrum of intermediate **3**.

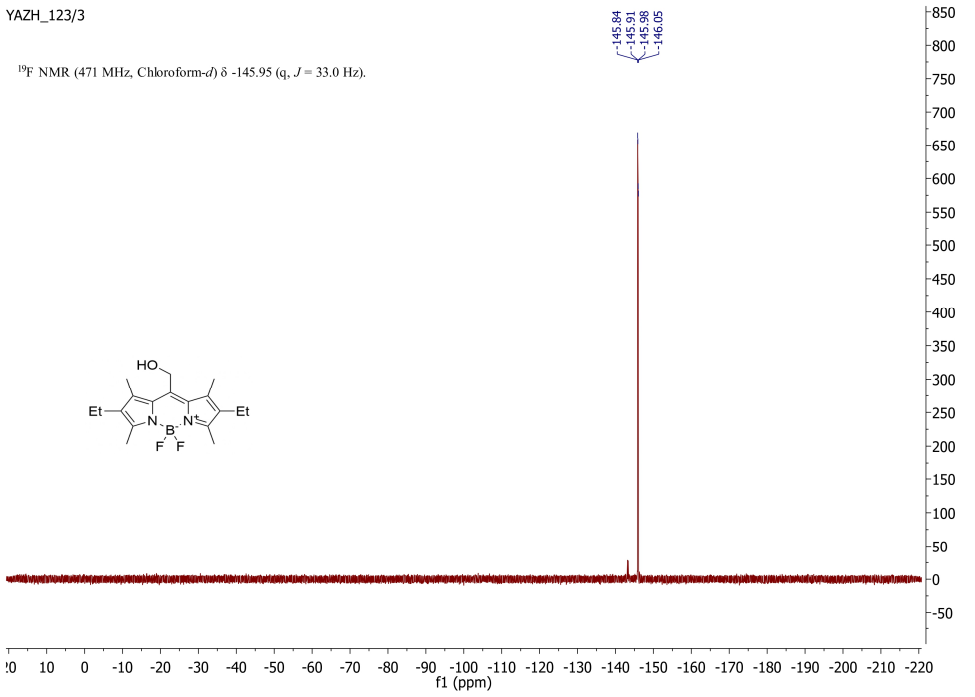

Figure S14. ^{19}F NMR spectrum of intermediate **3**.

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Report Format	: DefaultLCMS.lcr
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Processed by	: Admin
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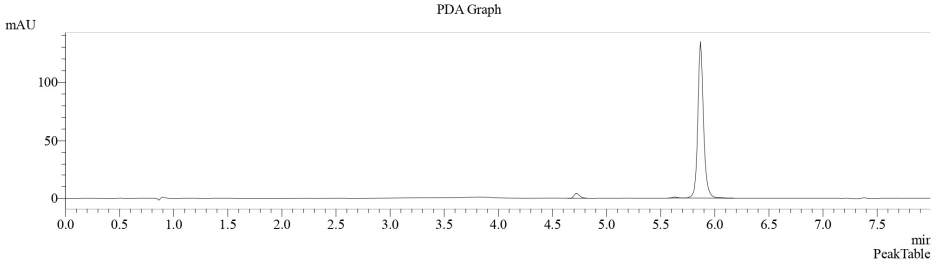

PeakTable

PDA Ch1 254nm 4nm

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MS Chromatogram

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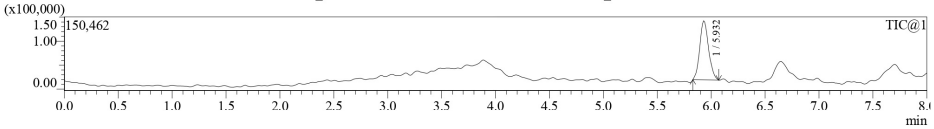

MS Spectrum Graph

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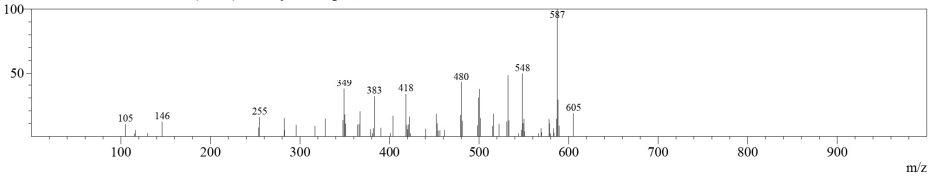

MS Spectrum Table

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1	105.00	1355	9.74				10	295.75	1249	8.98			
2	115.00	315	2.26				11	316.70	1156	8.31			
3	116.00	692	4.98				12	328.15	1936	13.92			
4	129.95	364	2.62				13	348.05	1766	12.70			
5	145.95	1602	11.52				14	349.10	5183	37.26			
6	253.80	979	7.04				15	350.05	2376	17.08			
7	254.80	2100	15.10				16	351.00	1395	10.03			
8	282.30	1985	14.27				17	364.05	1274	9.16			
9	282.75	689	4.95				18	365.95	1334	9.59			

#	m/z	Abs.Inten.	Rel.Inten.	Charge	Polarity	Monoisotopic	#	m/z	Abs.Inten.	Rel.Inten.	Charge	Polarity	Monoisotopic
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20	379.00	810	5.82				46	515.15	1135	8.16			
21	380.95	369	2.65				47	516.20	2468	17.74			
22	381.95	898	6.46				48	522.25	1365	9.81			
23	383.15	4365	31.38				49	531.05	1604	11.53			
24	390.35	930	6.69				50	532.35	6752	48.54			
25	401.10	365	2.62				51	533.30	1746	12.55			
26	403.90	2237	16.08				52	544.20	340	2.44			
27	418.35	4610	33.14				53	547.35	714	5.13			
28	419.40	1235	8.88				54	548.35	6934	49.85			
29	420.35	789	5.67				55	549.20	1446	10.40			
30	421.40	1307	9.40				56	550.25	1906	13.70			
31	422.25	2152	15.47				57	550.65	556	4.00			
32	423.30	359	2.58				58	566.15	338	2.43			
33	440.35	829	5.96				59	569.25	904	6.50			
34	452.45	2446	17.59				60	569.65	460	3.31			
35	453.30	1412	10.15				61	578.20	1882	13.53			
36	454.30	640	4.60				62	578.60	1421	10.22			
37	456.35	670	4.82				63	579.60	349	2.51			
38	461.35	695	5.00				64	583.20	880	6.33			
39	479.25	2322	16.69				65	583.60	409	2.94			
40	480.30	5931	42.64				66	586.45	1922	13.82			
41	481.20	1707	12.27				67	587.30	13909	100.00			
42	498.20	1206	8.67				68	588.20	3984	28.64			
43	499.20	4212	30.28				69	589.30	1180	8.48			
44	500.30	5141	36.96				70	605.30	2514	18.07			

Figure S15. LCMS chromatogram of intermediate **4**.

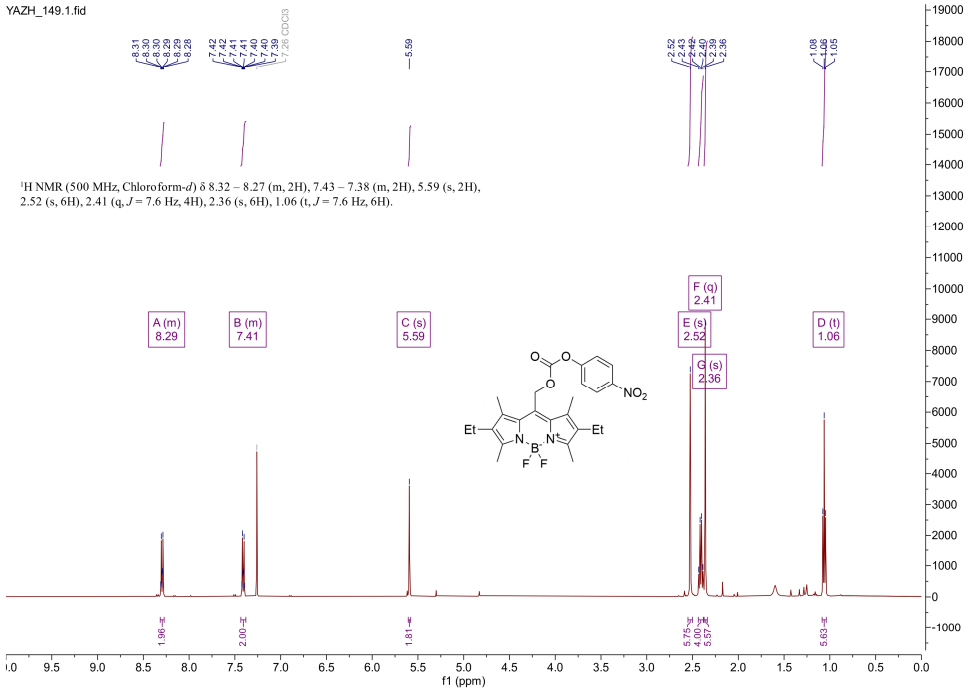

Figure S16. ^{1}H NMR spectrum of intermediate **4**.

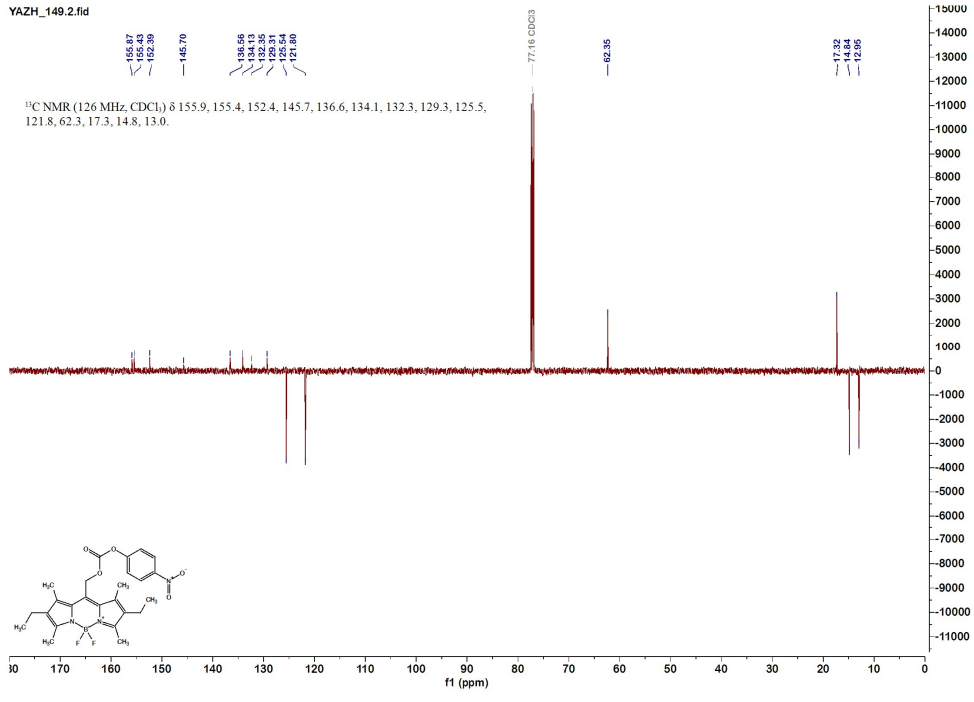

Figure S17. ^{13}C NMR spectrum of intermediate **4**.

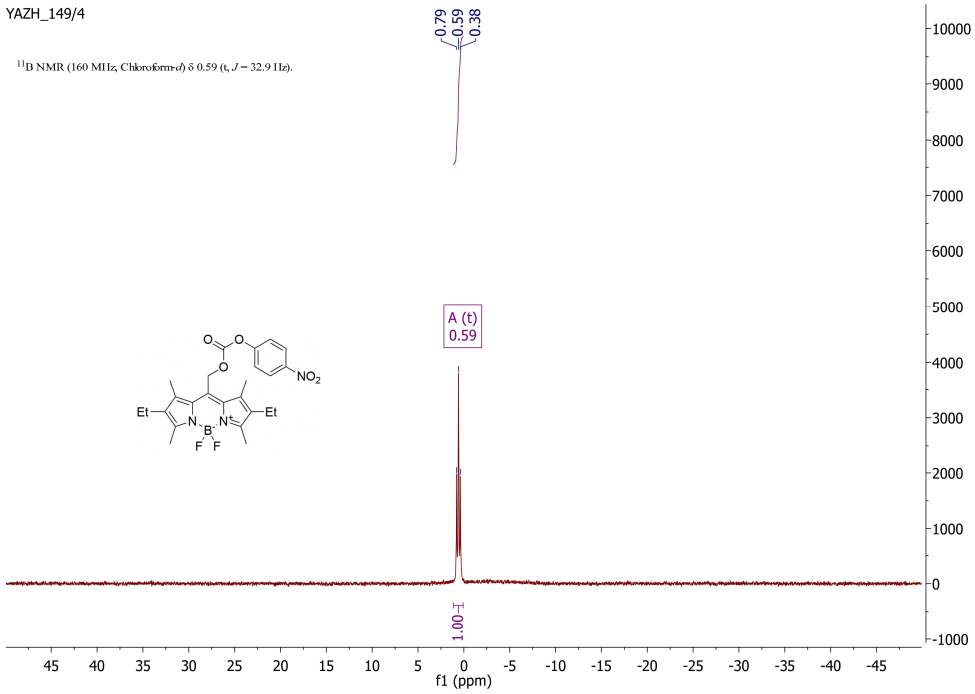

Figure S18. ^{11}B NMR spectrum of intermediate **4**.

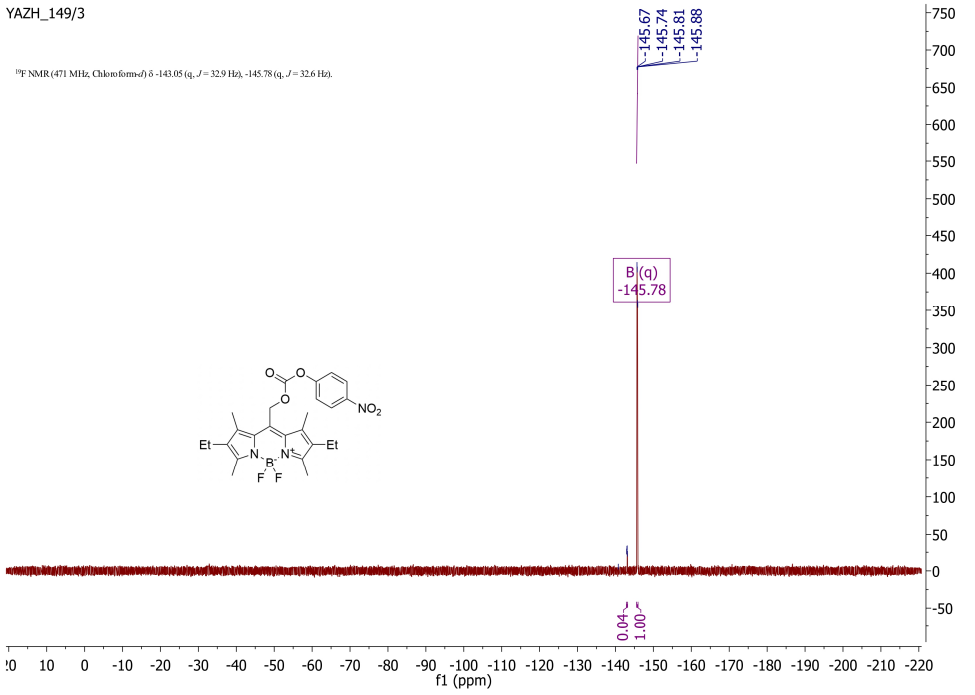

Figure S19. ^{19}F NMR spectrum of intermediate **4**.

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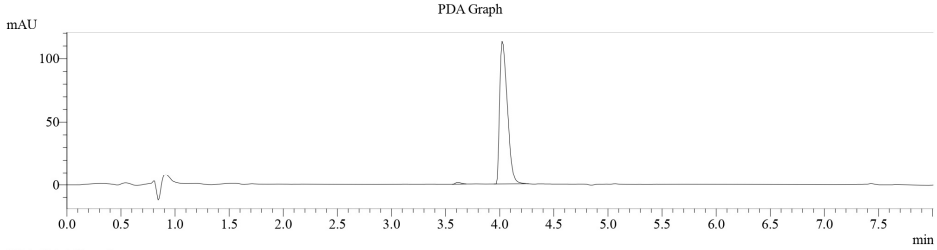
PDA Graph



PDA Ch1 254nm 4nm

Peak#	Name	Ret. Time	Area	Area %
1		3.607	5041	0.894
2		4.019	558949	99.106

MS Chromatogram

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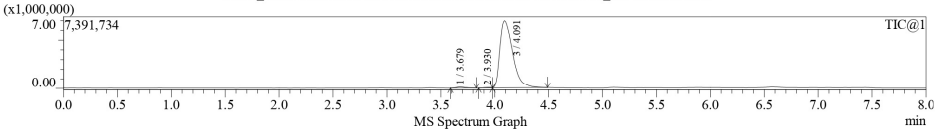
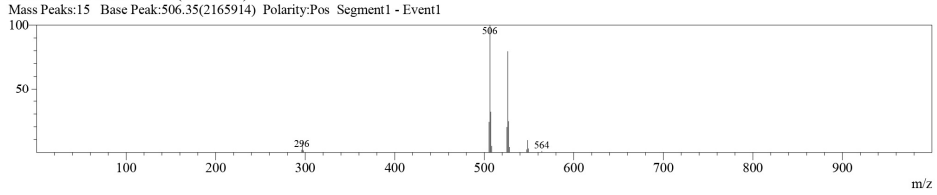


MS Spectrum Graph

#:1 Ret.Time:Averaged 4.080-4.100(Scan#:409-411)
BG Mode:Calc 3.980<->4.490(399<->450)
Mass Peaks:15 Base Peak:506.35(2165914) Polarity:Pos Segment1 - Event1



MS Spectrum Table

#:1 Ret.Time:
BG Mode:Calc 3.980<->4.490(399<->450)
Mass Peaks:15 Base Peak:506.35(2165914) Polarity:Pos Segment1 - Event1

#	m/z	Abs.Inten.	Rel.Inten.	Charge	Polarity	Monoisotopic	#	m/z	Abs.Inten.	Rel.Inten.	Charge	Polarity	Monoisotopic
1	296.20	48018	2.22				9	526.35	1725061	79.65			
2	297.10	182775	8.44				10	527.35	528346	24.39			
3	298.10	35108	1.62				11	528.35	91724	4.23			
4	505.30	516176	23.83				12	547.30	50778	2.34			
5	506.35	2165914	100.00				13	548.30	208443	9.62			
6	507.30	686504	31.70				14	549.35	67822	3.13			
7	508.35	111055	5.13				15	564.30	24446	1.13			
8	525.35	428810	19.80										

Figure S20. LCMS chromatogram of final compound 5.

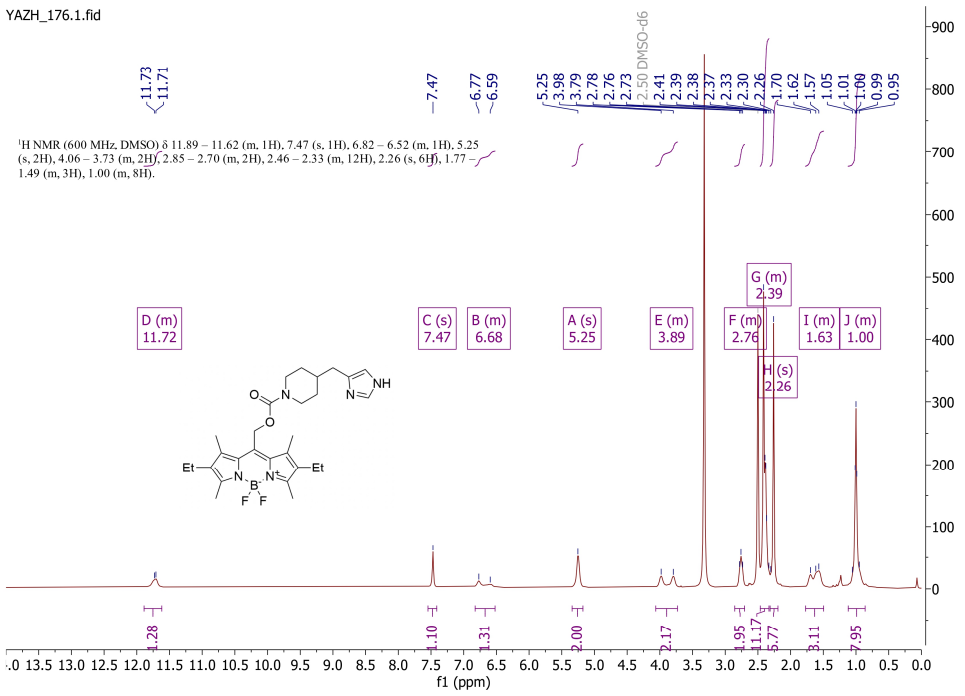


Figure S21. ^{1}H NMR spectrum of final compound **5**.

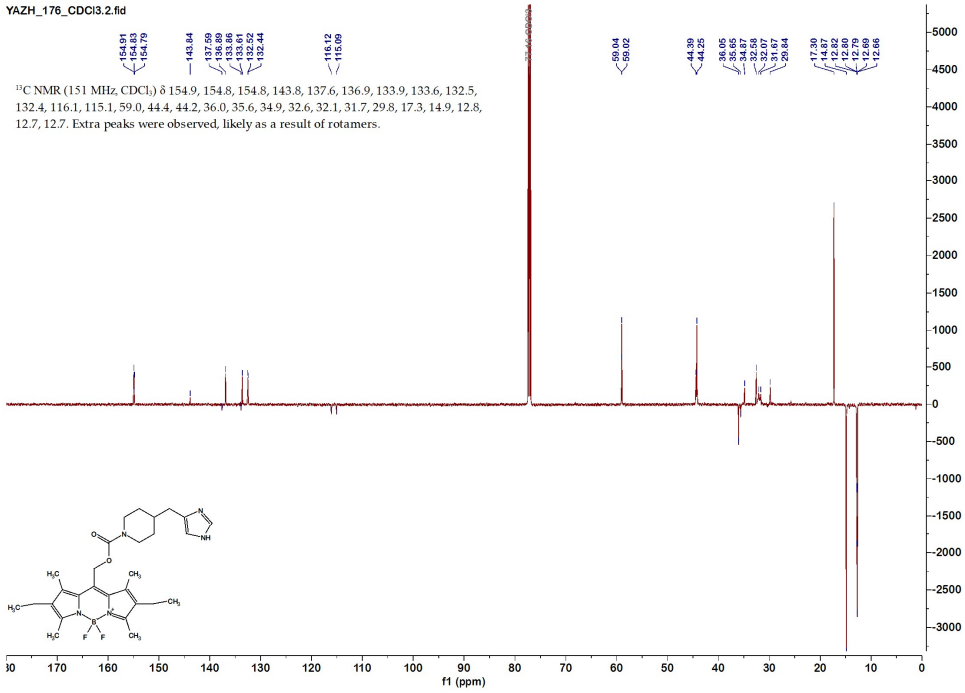


Figure S22. ^{13}C NMR spectrum of final compound **5**.

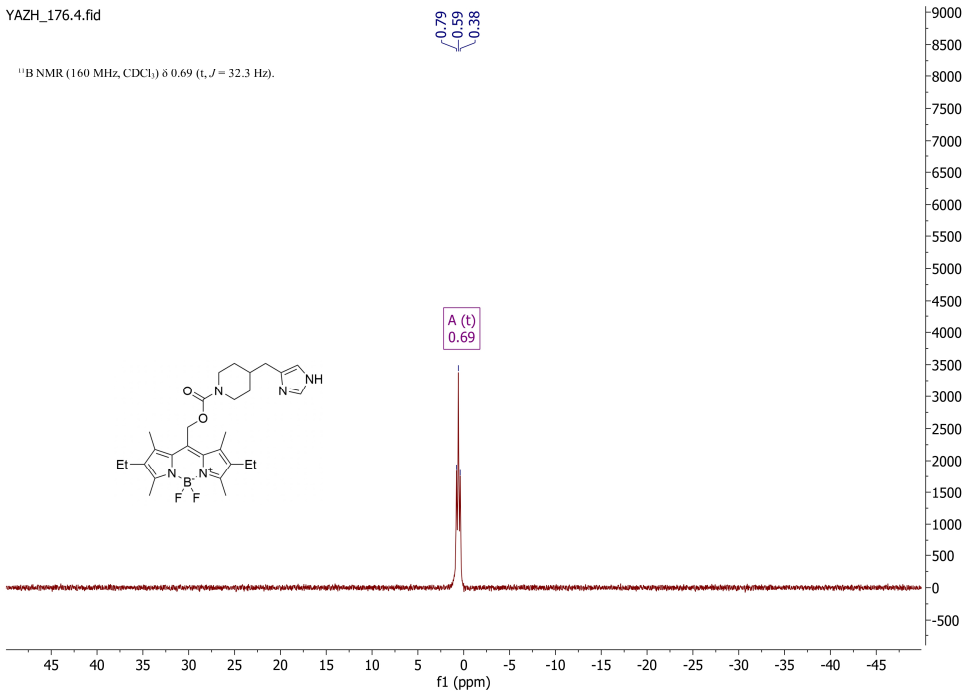


Figure S23. ^{11}B NMR spectrum of final compound **5**.

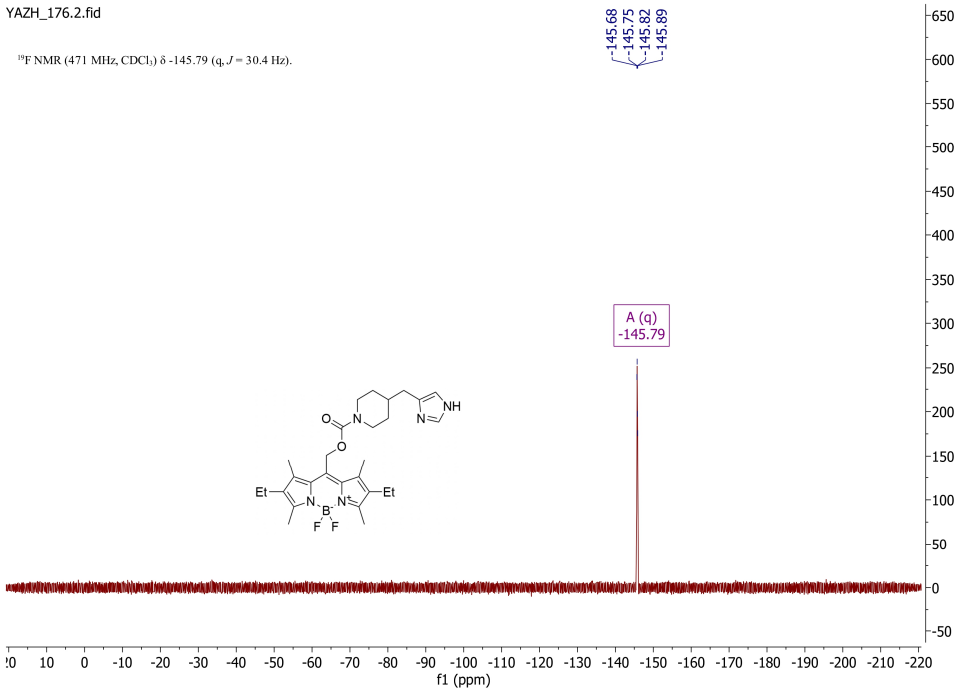


Figure S24. ^{19}F NMR spectrum of final compound **5**.

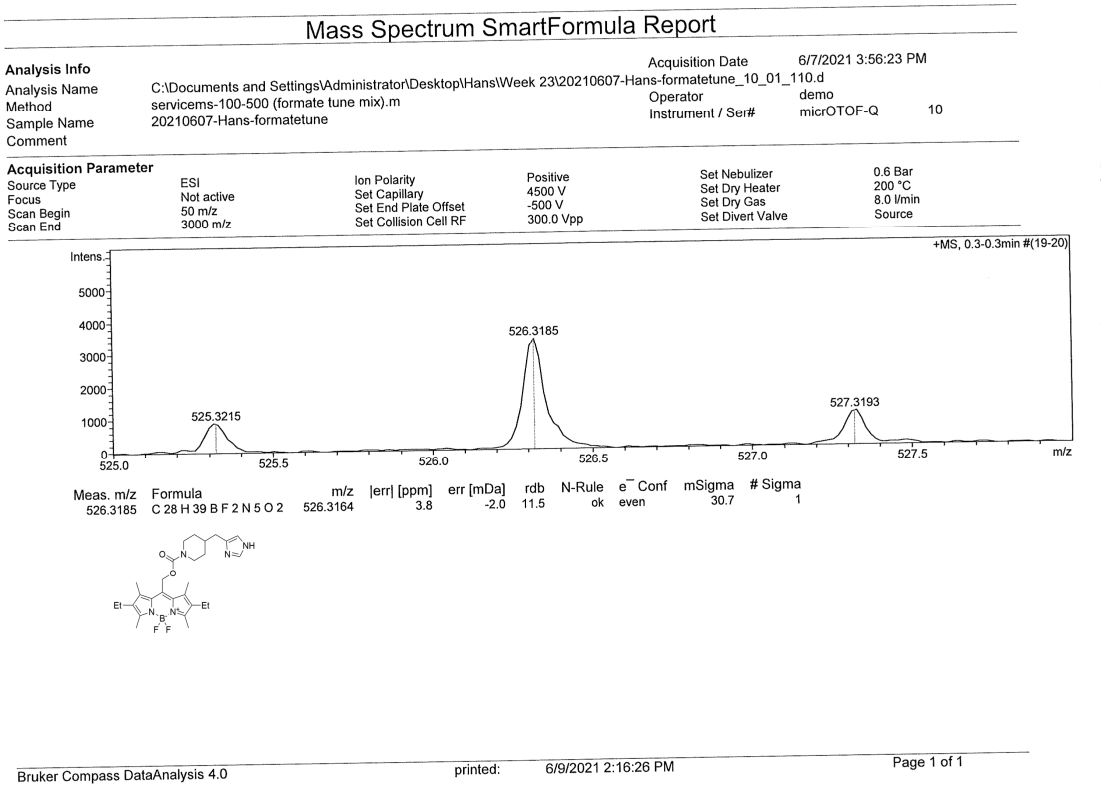
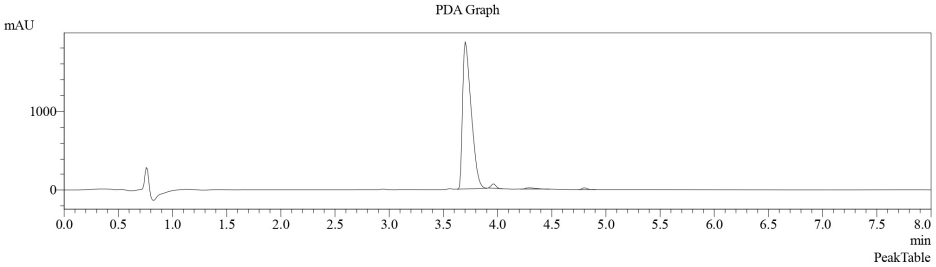


Figure S25. HRMS spectrum of final compound **5**.

Acquired by	: Admin
Date Acquired	: 23/6/2021 10:17:40 AM
Sample Name	: YAZH_181_purified
Sample ID	:
Tray#	: 1
Vial#	: 5
Injection Volume	: 20
Data File	: C:\LabSolutions\Data\2021\2021-wk25\YAZH_181_purified.lcd
Background File	: blanco 23062021.lcd
Method File	: Method SCAN ACID standard.lcm
Report Format	: DefaultLCMS.lcr
Tuning File	: C:\LabSolutions\Tuning\tuning 15042021.lct
Processed by	: Admin
Modified Date	: 23/6/2021 12:16:07 PM

PDA Graph



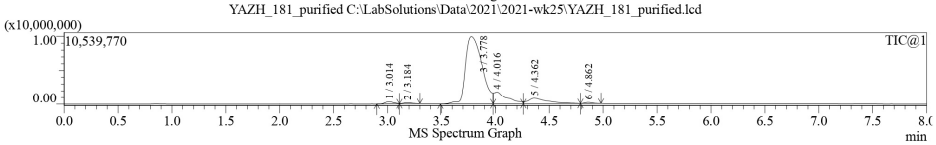
PeakTable

PDA Ch1 230nm 4nm

Peak#	Name	Ret. Time	Area	Area %
1		3.698	10273222	96.849
2		3.957	163161	1.538
3		4.284	101552	0.957
4		4.796	69532	0.655

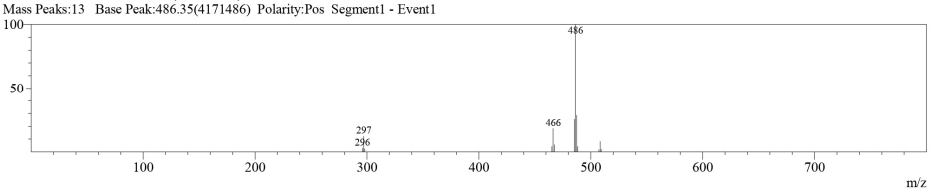
MS Chromatogram

YAZH_181_purified C:\LabSolutions\Data\2021\2021-wk25\YAZH_181_purified.lcd



MS Spectrum Graph

#:1 Ret.Time:Averaged 3.770-3.790(Scan#:378-380)
BG Mode:Calc 3.490<->3.980(350<->399)
Mass Peaks:13 Base Peak:486.35(4171486) Polarity:Pos Segment1 - Event1



MS Spectrum Table

#:1 Ret.Time:
BG Mode:Calc 3.490<->3.980(350<->399)
Mass Peaks:13 Base Peak:486.35(4171486) Polarity:Pos Segment1 - Event1

#	m/z	Abs.Inten.	Rel.Inten.	Charge	Polarity	Monoisotopic	#	m/z	Abs.Inten.	Rel.Inten.	Charge	Polarity	Monoisotopic
1	296.10	124613	2.99				8	486.35	4171486	100.00			
2	297.10	510932	12.25				9	487.35	1196218	28.68			
3	298.15	100905	2.42				10	488.35	182102	4.37			
4	465.30	182931	4.39				11	507.30	77071	1.85			
5	466.30	767534	18.40				12	508.35	347791	8.34			
6	467.30	237233	5.69				13	509.30	90988	2.18			
7	485.30	1065553	25.54										

Figure S26. LCMS chromatogram of final compound **6**.

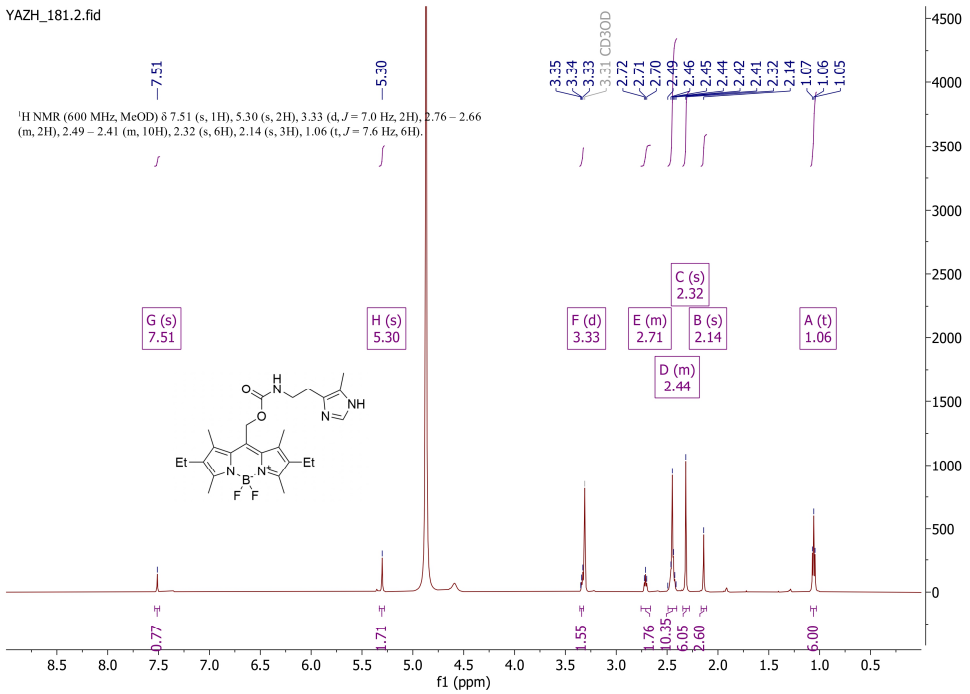


Figure S27. ^{1}H NMR spectrum of final compound **6**.

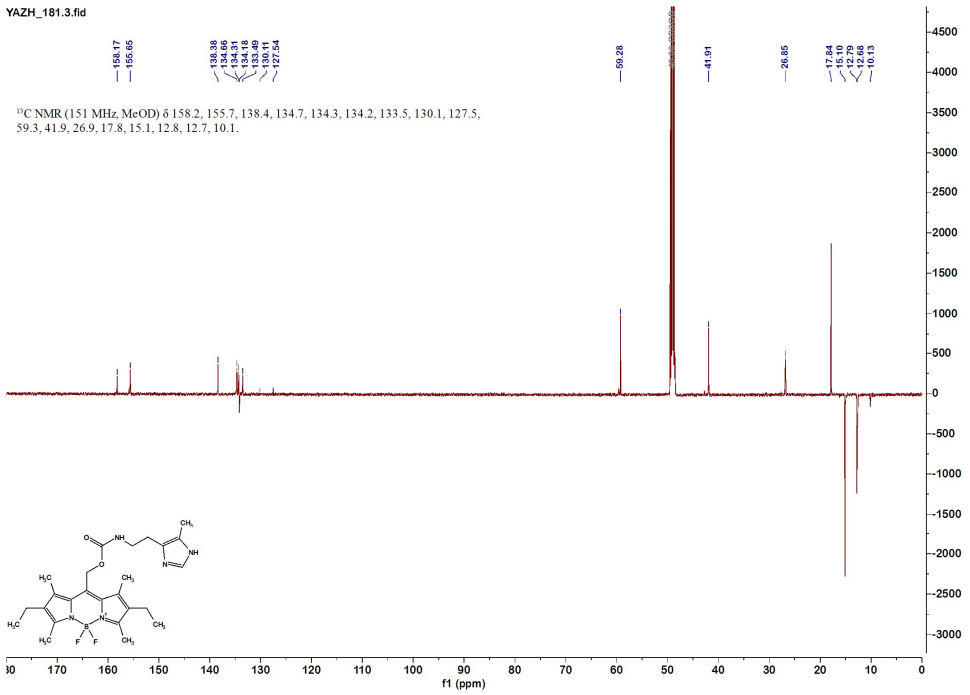


Figure S28. ^{13}C NMR spectrum of final compound **6**.

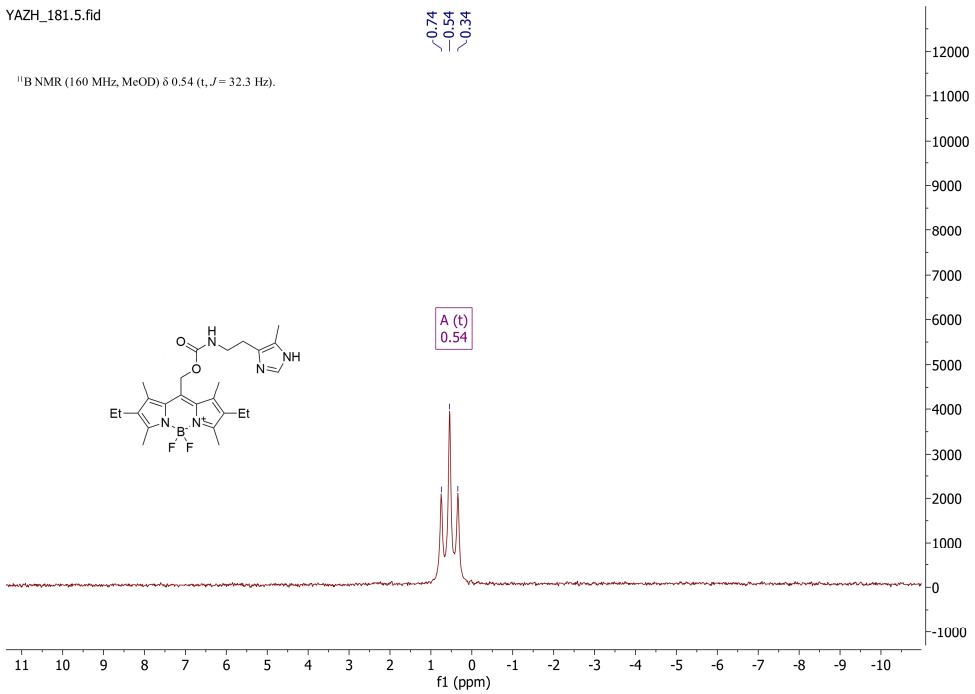


Figure S29. ^{11}B NMR spectrum of final compound **6**.

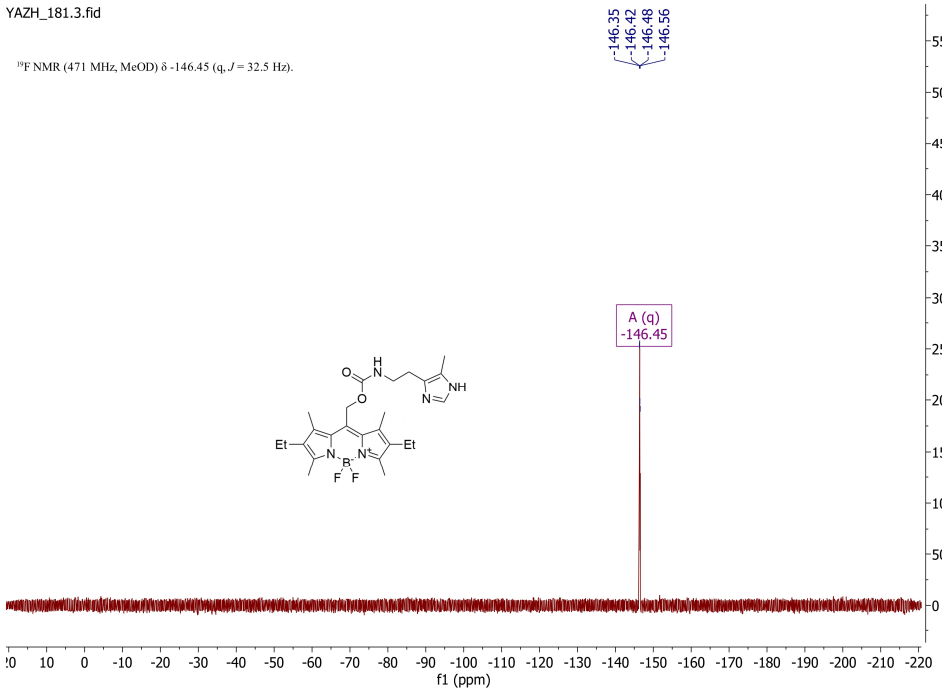


Figure S30. ^{19}F NMR spectrum of final compound **6**.

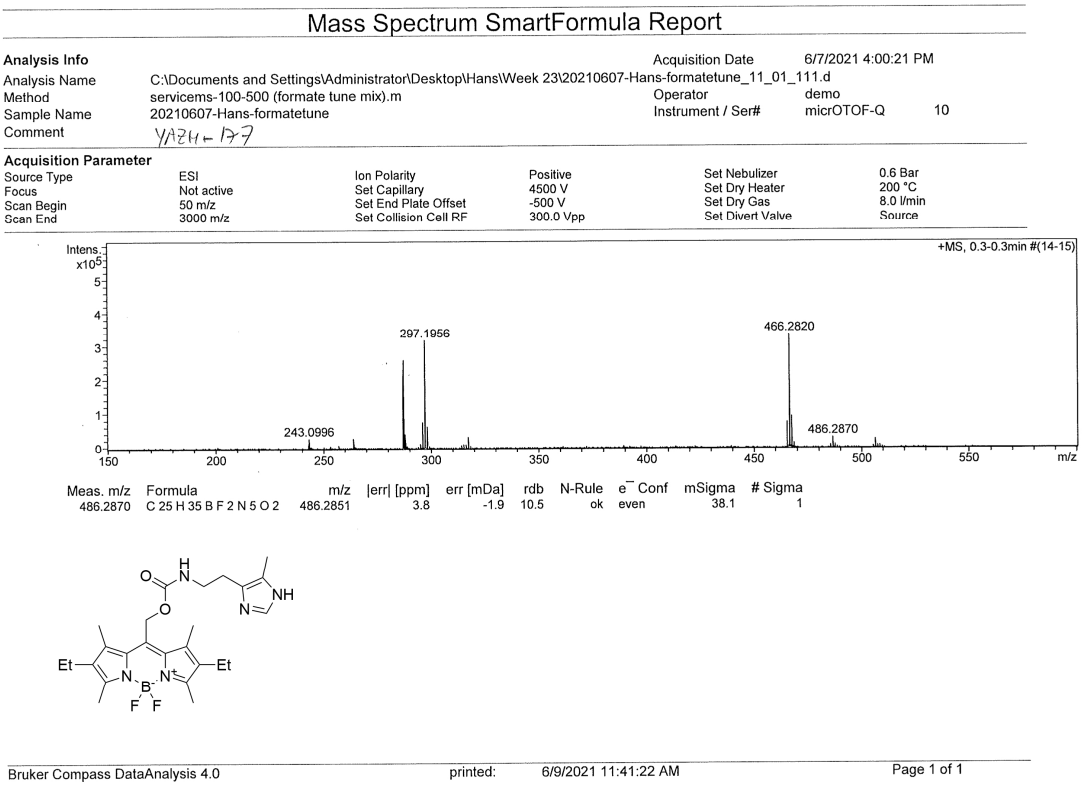


Figure S31. HRMS spectrum of final compound **6**.