Supplementary Information

Development of water-soluble phenazine-2,3-diol-based photosensitizers for singlet oxygen generation

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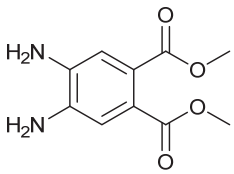
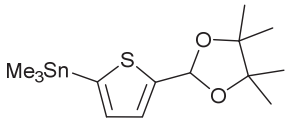
Synthesis



Compound **1**. A solution of dimethyl 4-amino-5-nitrophthalate (407 mg, 1.60 mmol) and ammonium chloride (428 mg, 8.00 mmol) in the mixture of ethanol (360 mL) and water (40 mL) was stirred at 80 ℃ with iron powder (447 mg, 8.00 mmol) overnight under N_2 atmosphere. After concentrating under reduced pressure, the residue was chromatographed on silica gel (methanol/dichloromethane = 1/19 as eluent) to give **1** as light brown solid (277 mg, 80% yield); m.p. 109–112 ℃; IR (ATR): = 3441, 3414, 3360, 3298, 3273, 3248, 2949, 1717, 1688, 1668, 1626, 1589, 1574, 1566, 1522 cm^{-1}; ^1H NMR (500 MHz, dimethyl sulfoxide (DMSO)-d_6): δ = 6.79 (s, 2H, aromatic), 5.22 (s, 4H, NH_2), 3.68 (s, 6H, CH_3) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ = 168.30, 137.09, 120.70, 113.60, 51.85 ppm; HRMS (ESI): *m/z* found 247.06876 $[M+Na]^+$, calculated for $C_{10}H_{12}O_4N_2Na$ $[M+Na]^+$: 247.06893.



Compound **2** was synthesized by the method in our previous study.[1]

Reference

1. S. Tsumura, K. Ohira, K. Hashimoto, K. Imato, and Y. Ooyama, *Mater. Chem. Front.*, 2020, **4**, 2762-2771.

NMR spectra

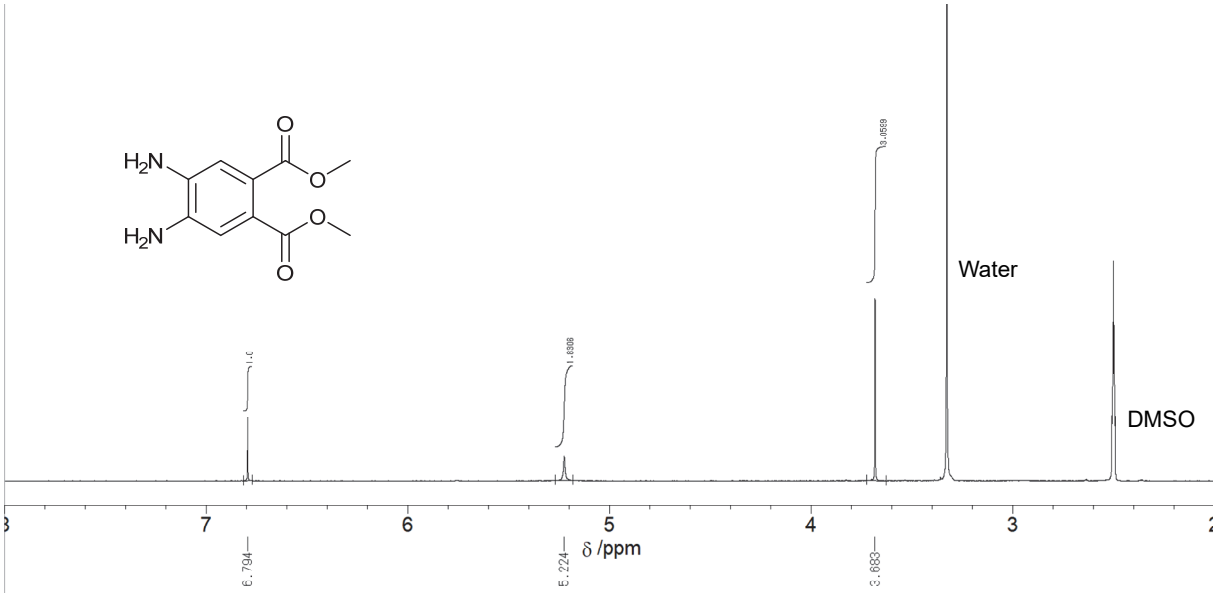


Fig. S1. ^{1}H NMR spectrum of **1** in DMSO-d_6.

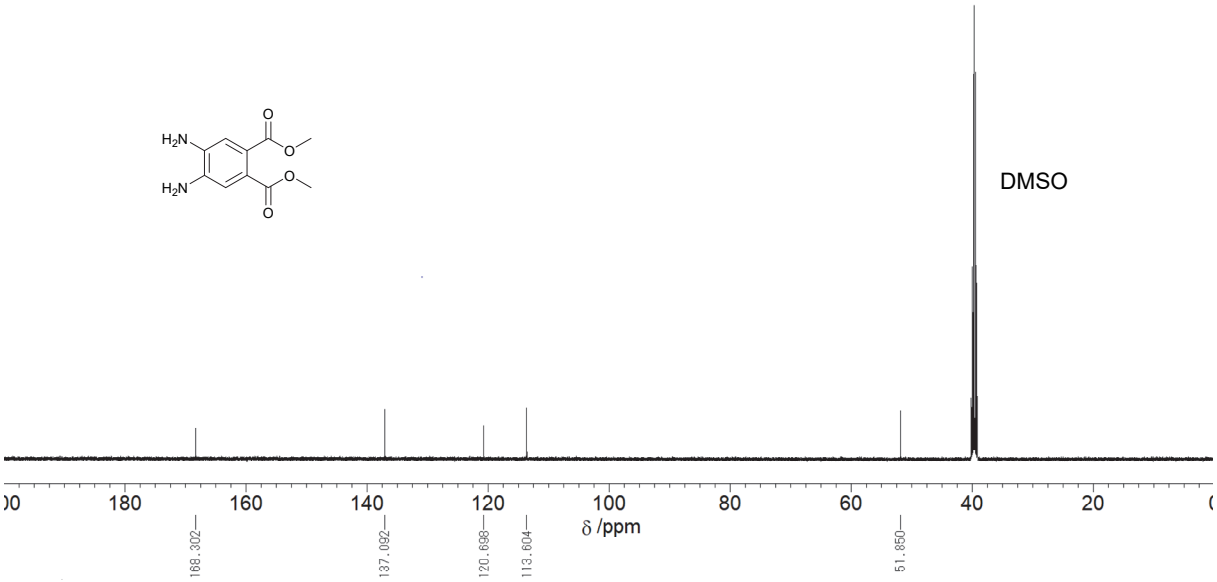


Fig. S2. ^{13}C NMR spectrum of **1** in DMSO-d_6

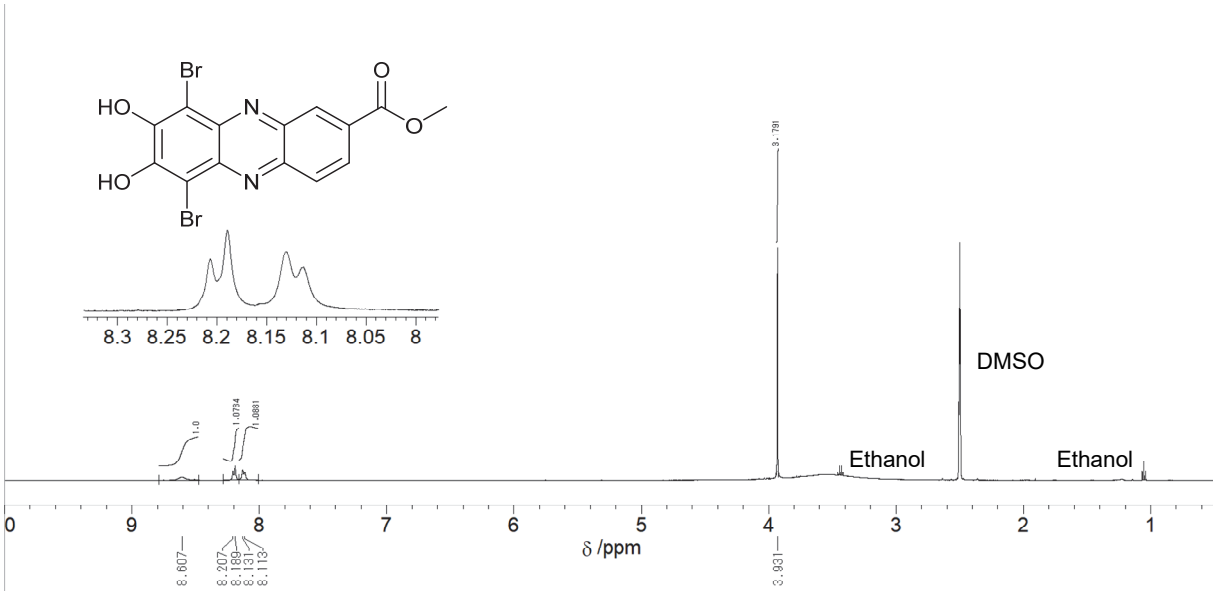


Fig. S3 ^{1}H NMR spectrum of **3** in DMSO-d_6.

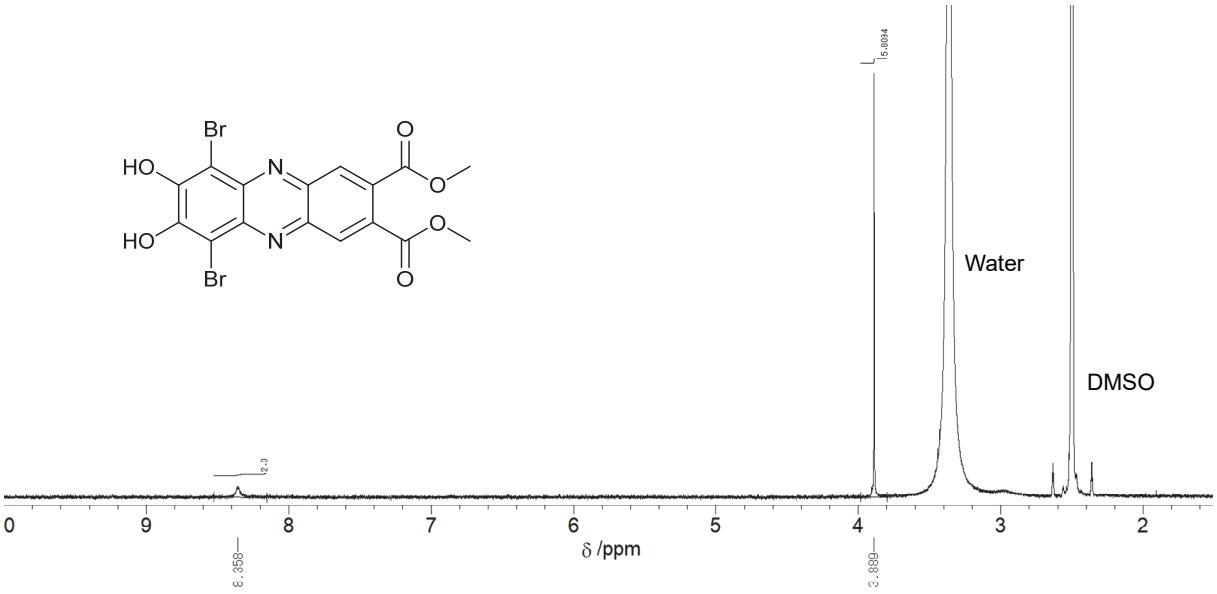


Fig. S4 ^{1}H NMR spectrum of **4** in DMSO-d_6.

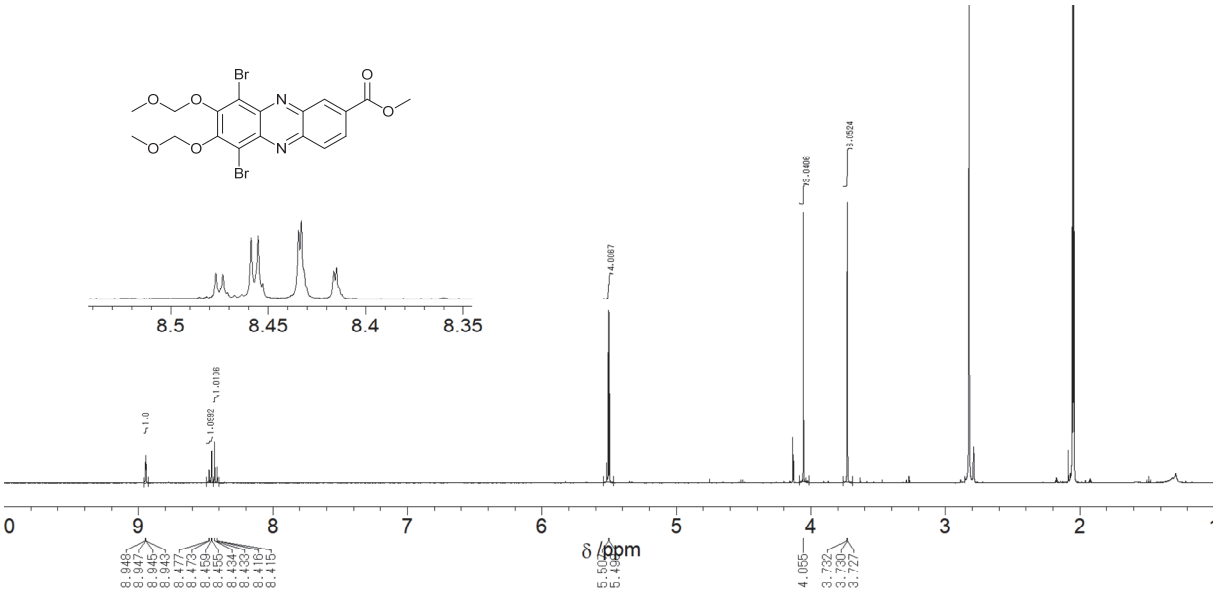


Fig. S5 ^{1}H NMR spectrum of **5** in acetone-d_6.

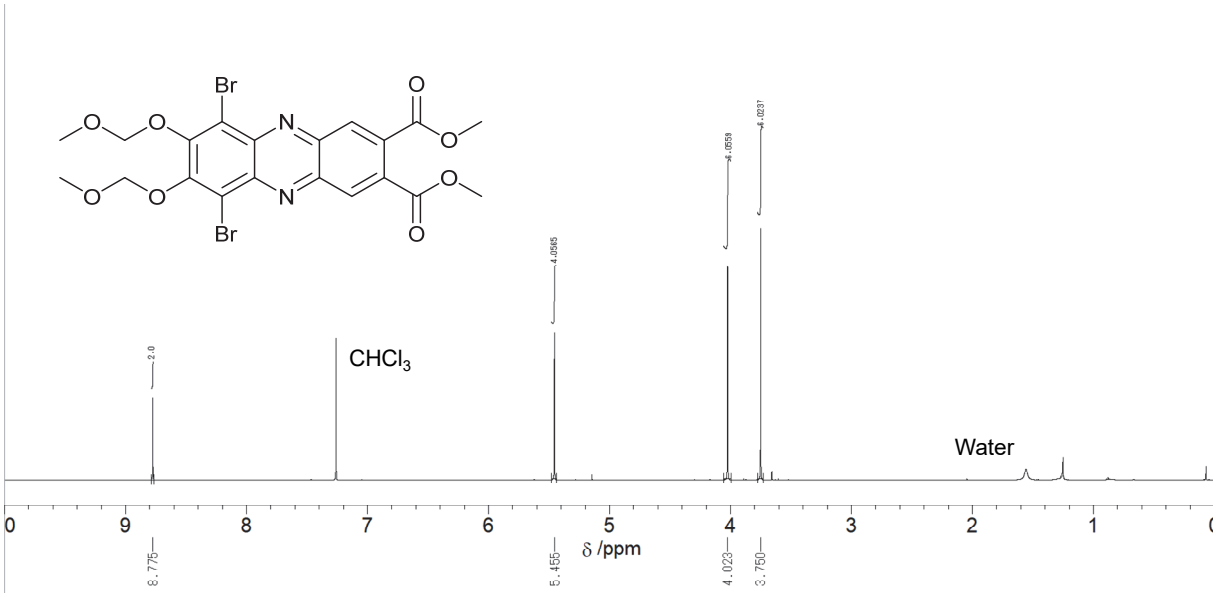


Fig. S6 ^{1}H NMR spectrum of **6** in $CDCl_3$.

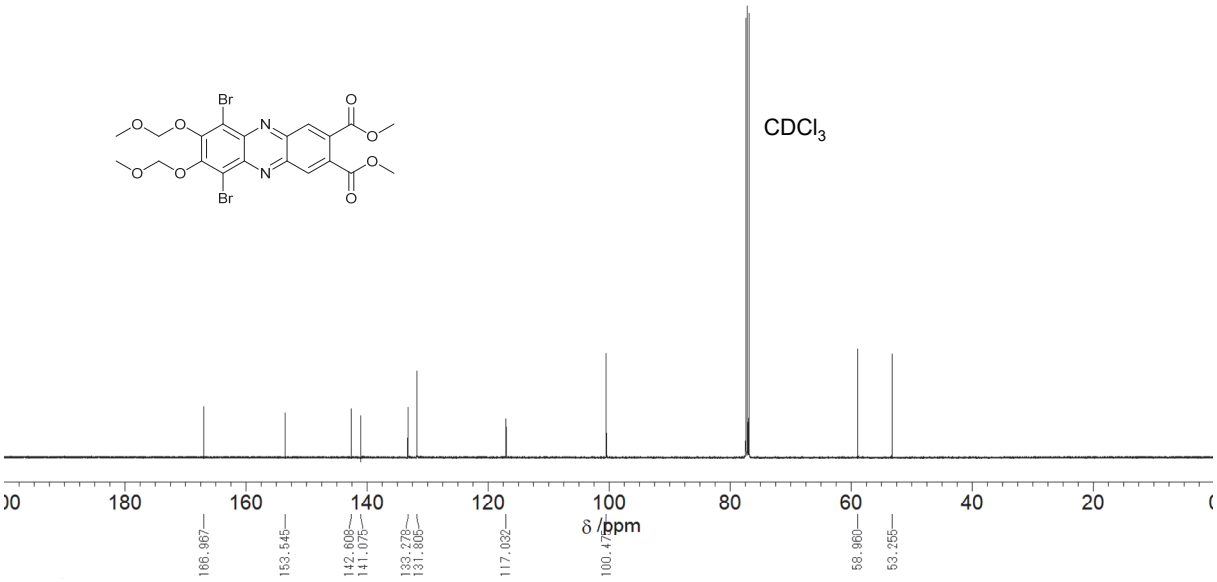


Fig. S7 ^{13}C NMR spectrum of **6** in $CDCl_3$.

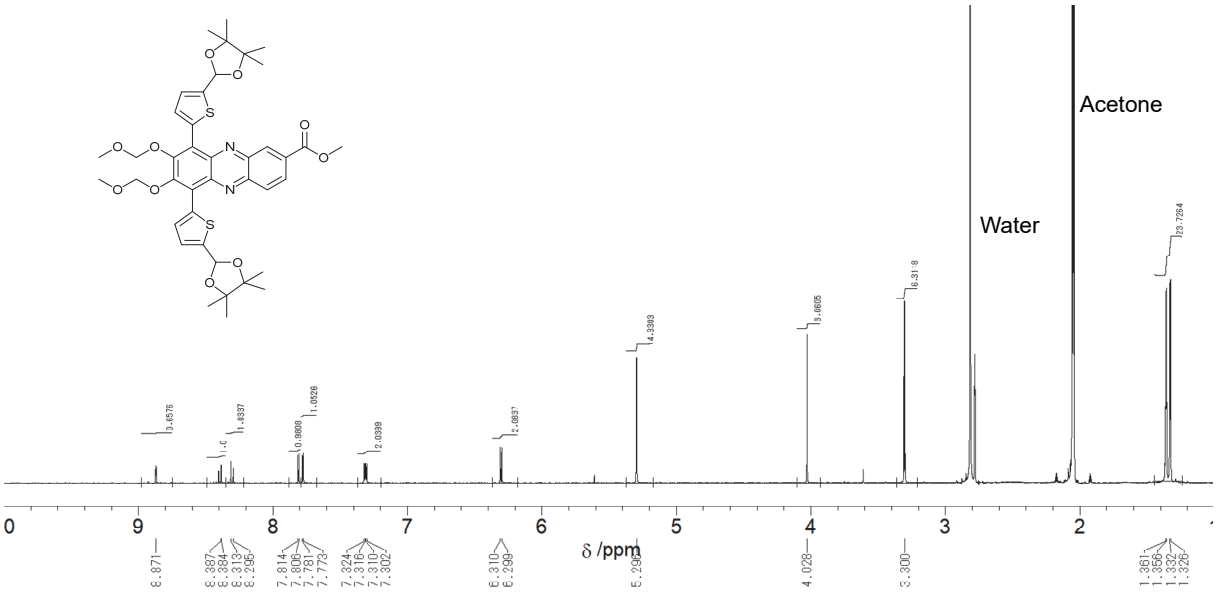


Fig. S8 ^{1}H NMR spectrum of **7** in acetone-d_6.

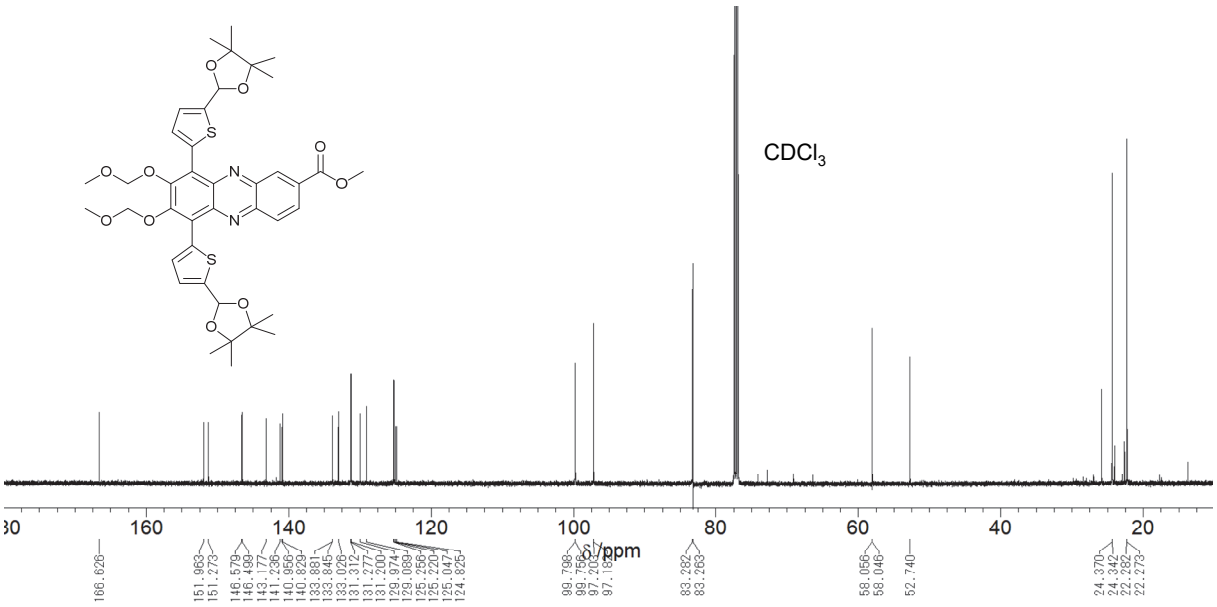


Fig. S9 ^{13}C NMR spectrum of **7** in $CDCl_3$.

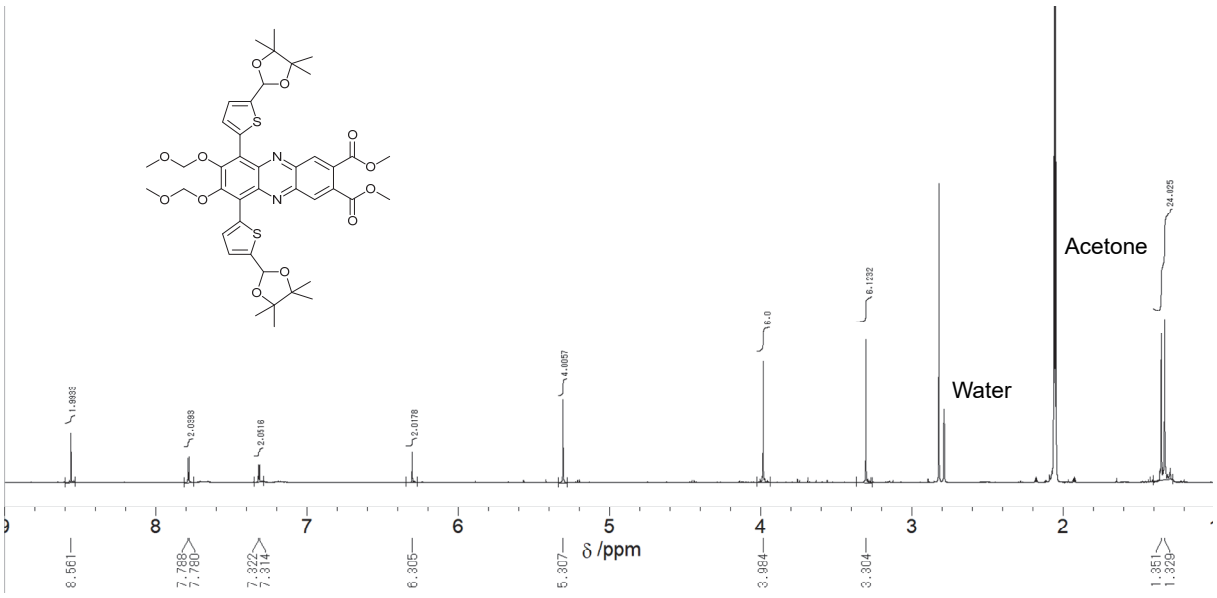


Fig. S10 ^{1}H NMR spectrum of **8** in acetone-d_6.

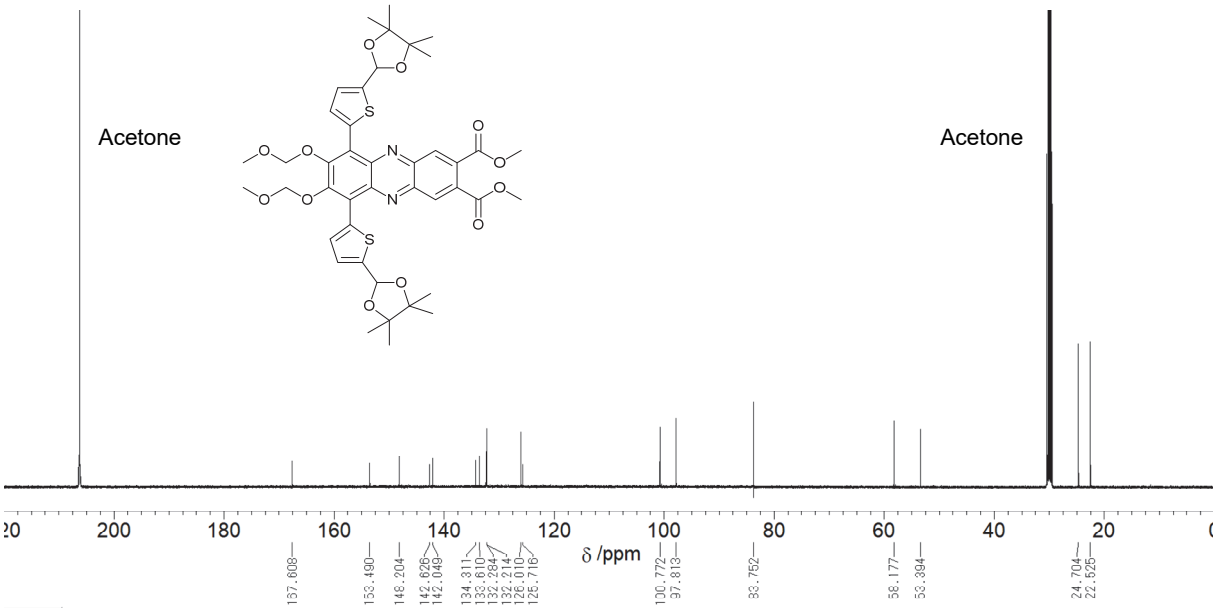


Fig. S11 ^{13}C NMR spectrum of **8** in acetone-d_6.

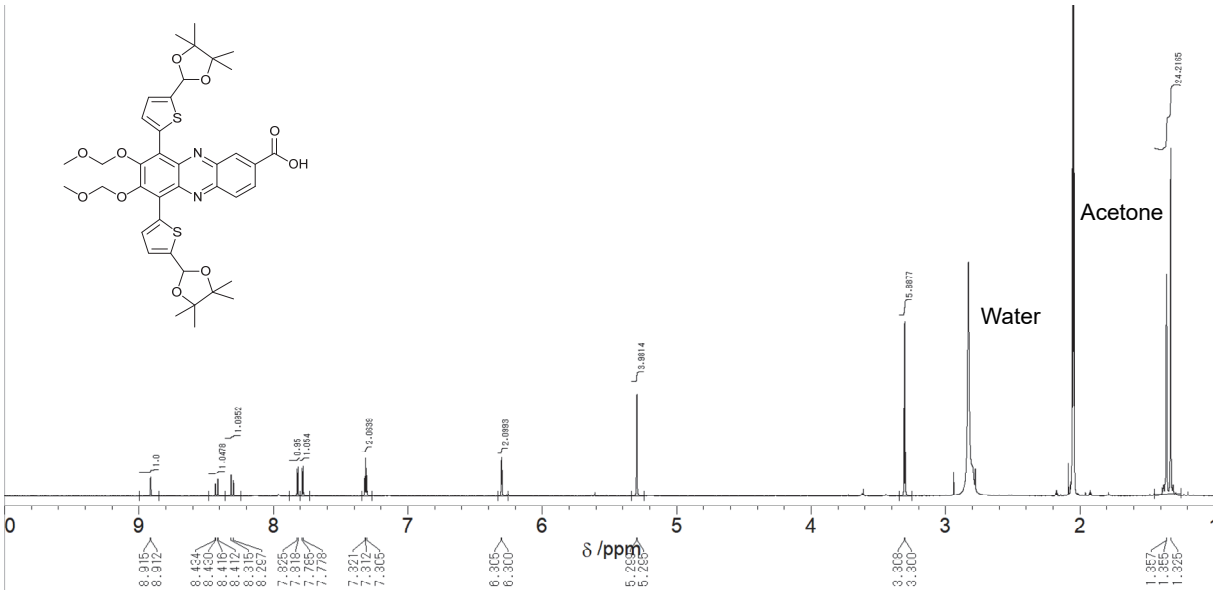


Fig. S12 ^{1}H NMR spectrum of **9** in acetone-d_6.

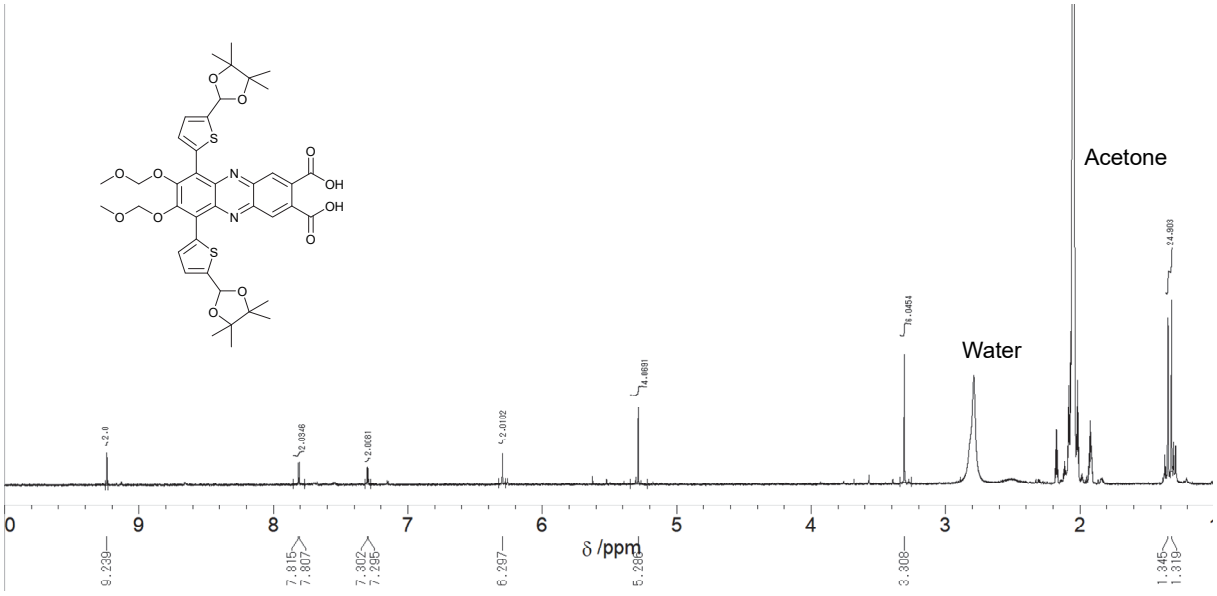


Fig. S13 ^{1}H NMR spectrum of **10** in acetone-d_6.

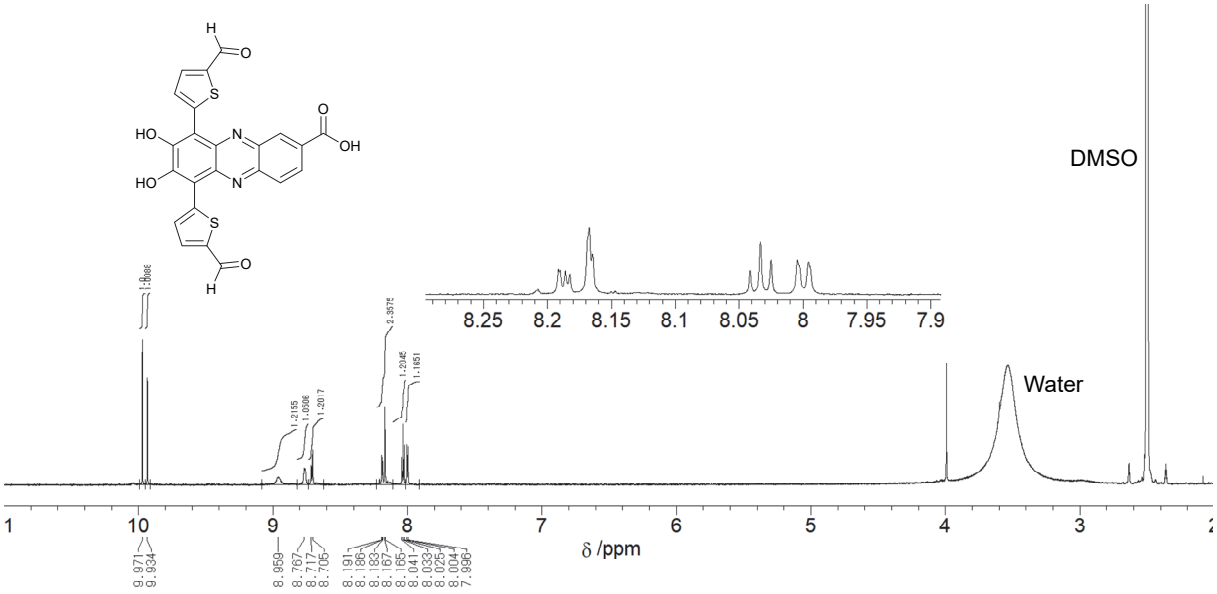


Fig. S14 ^{1}H NMR spectrum of **KY-1** in DMSO-d_6.

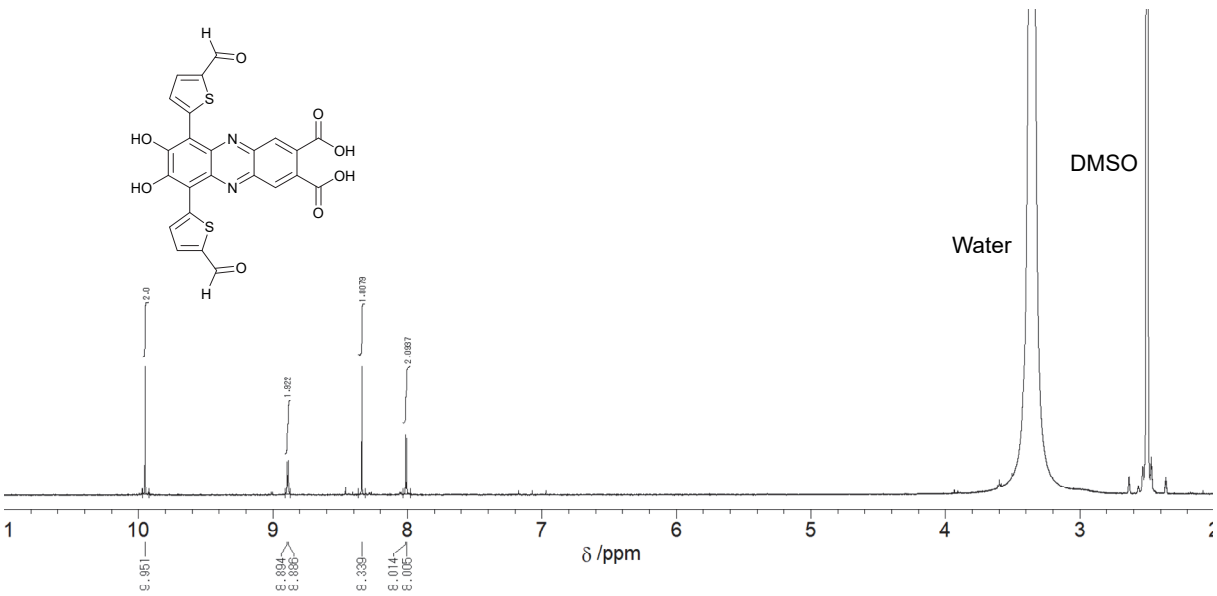


Fig. S15 [1]H NMR spectrum of **KY-2** in DMSO-d_6.

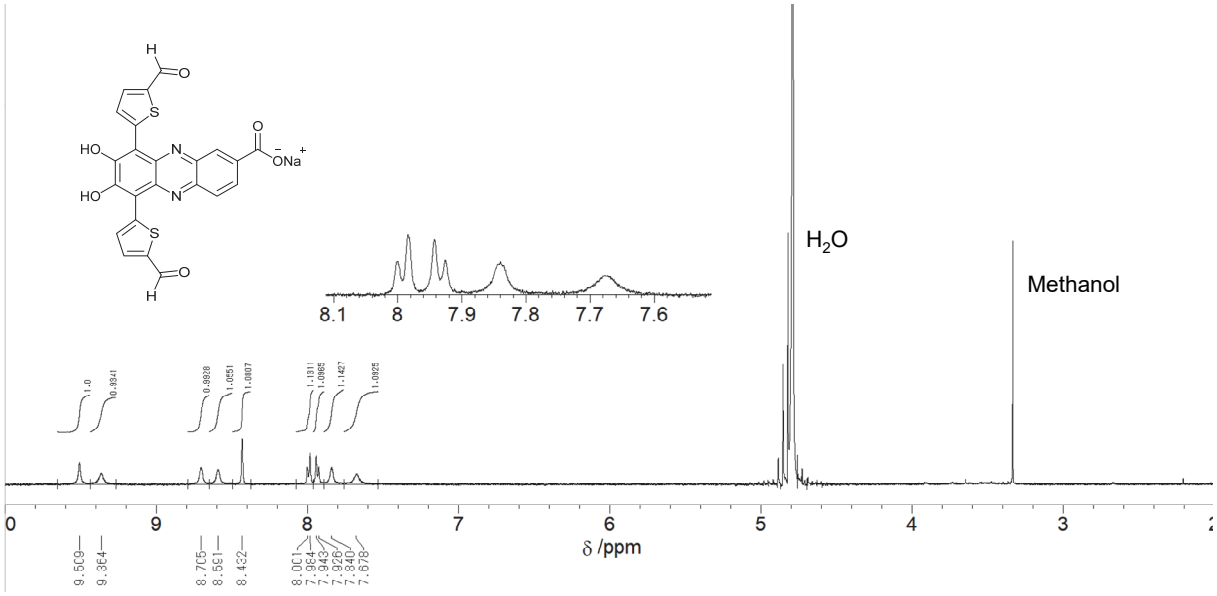


Fig. S16 [1]H NMR spectrum of **KY-1Na** in D_2O.

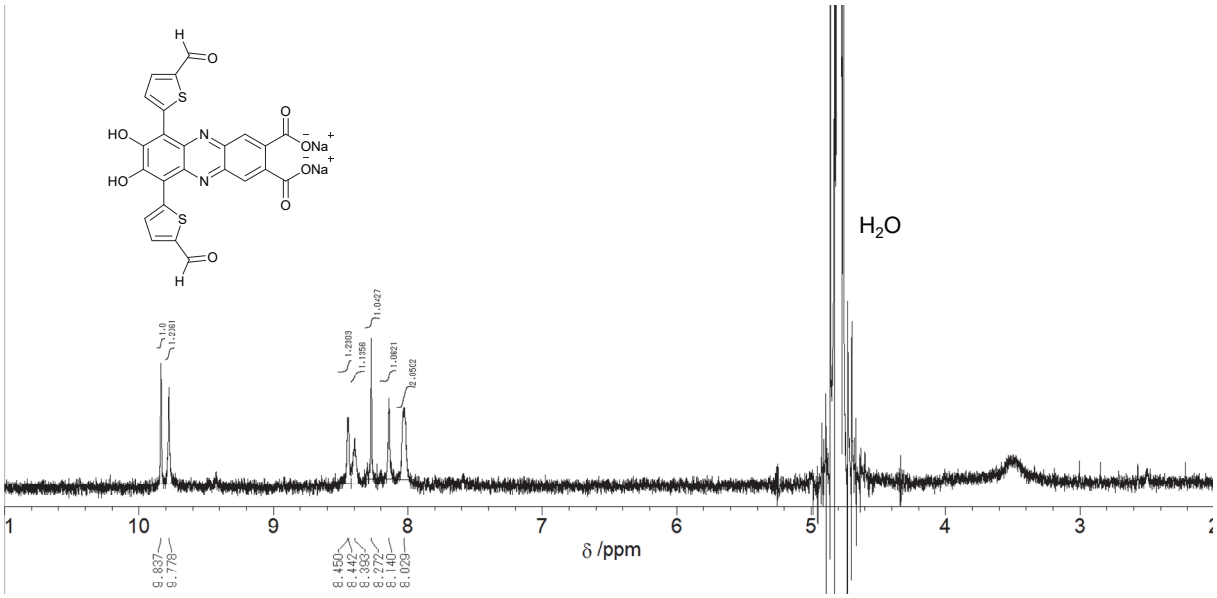


Fig. S17 ^{1}H NMR spectrum of **KY-2Na** in D_2O.

HRMS spectra

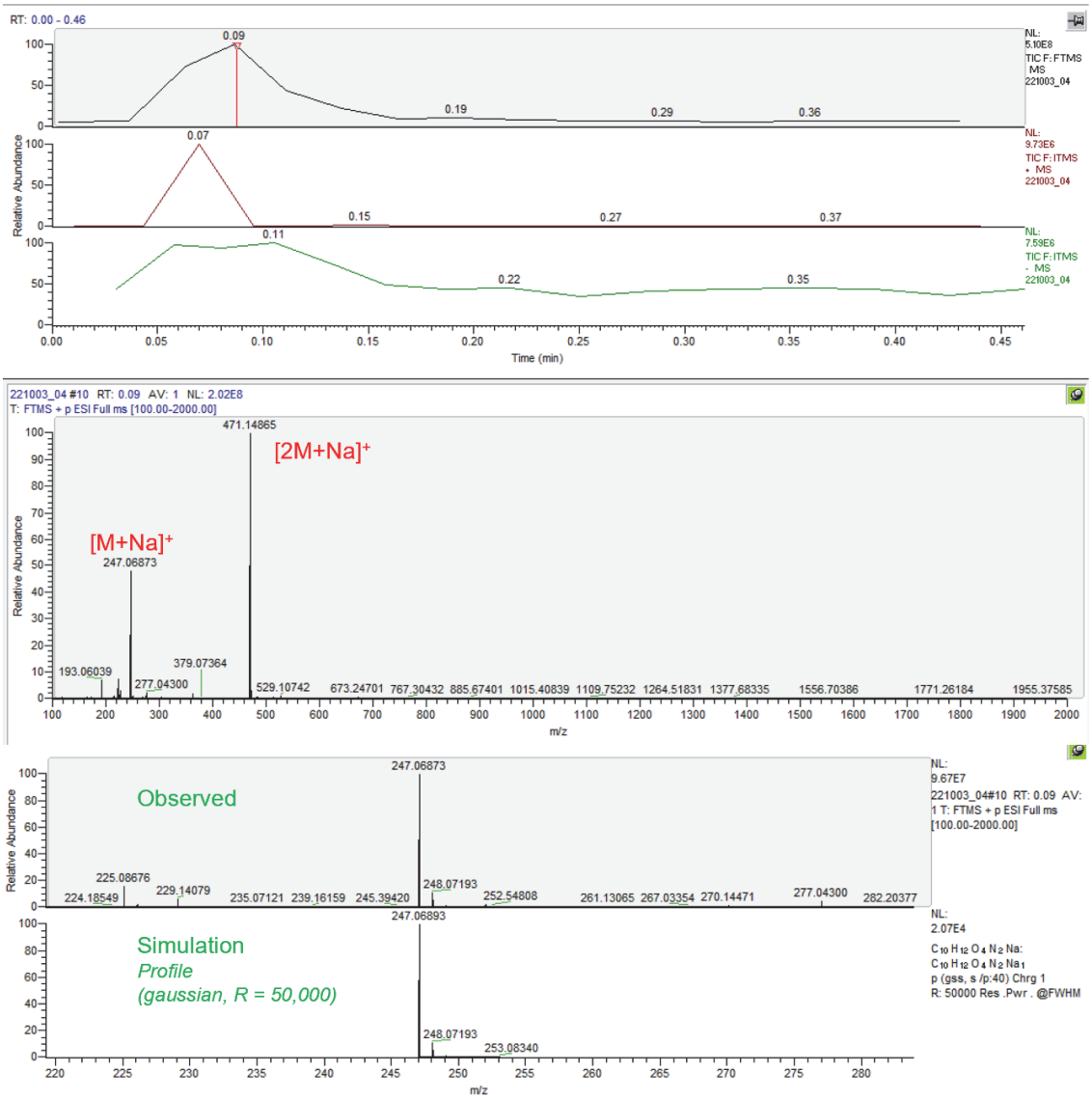


Fig. S18 HRMS spectrum of **1**.

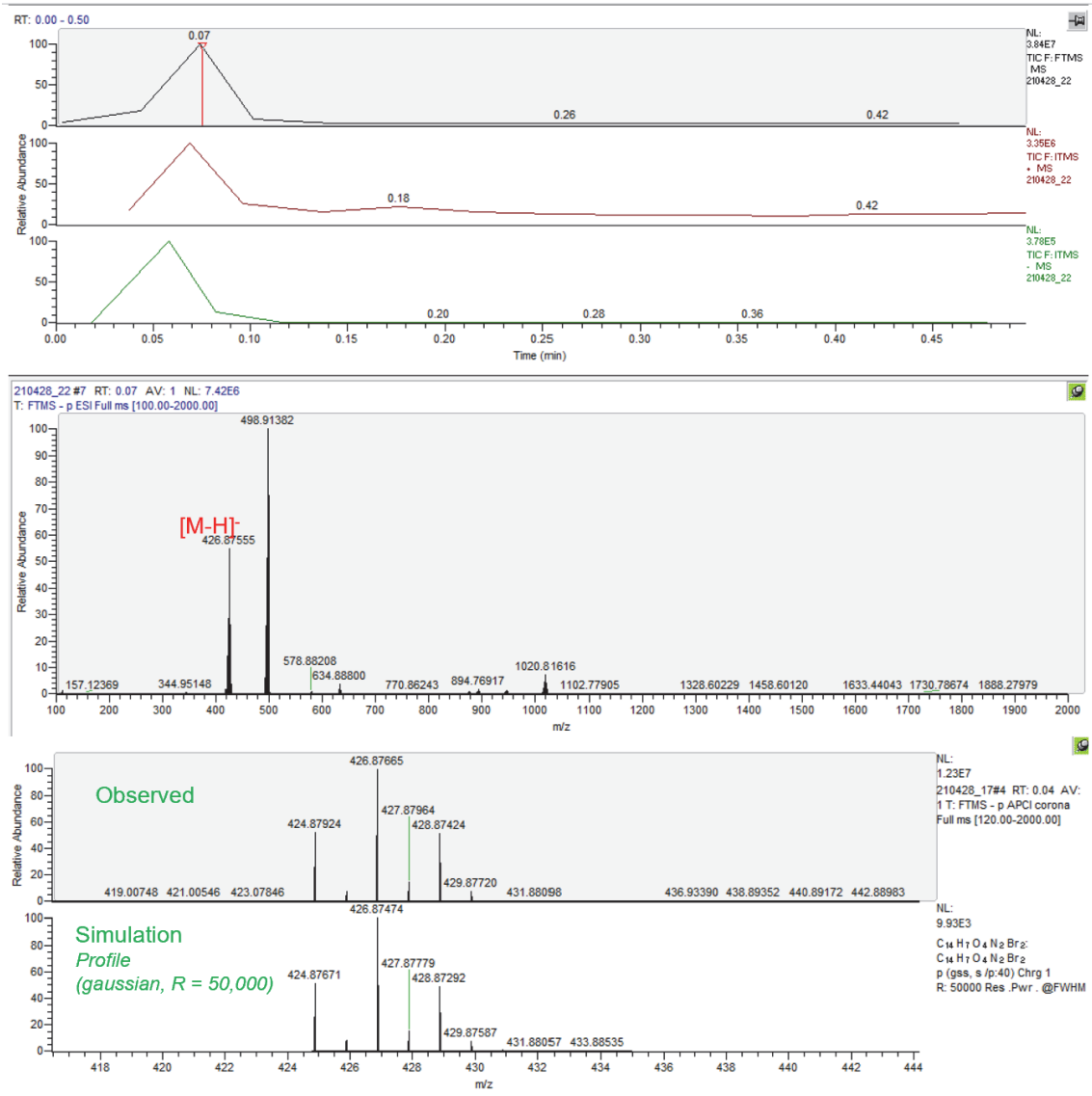


Fig. S19 HRMS spectrum of **3**.

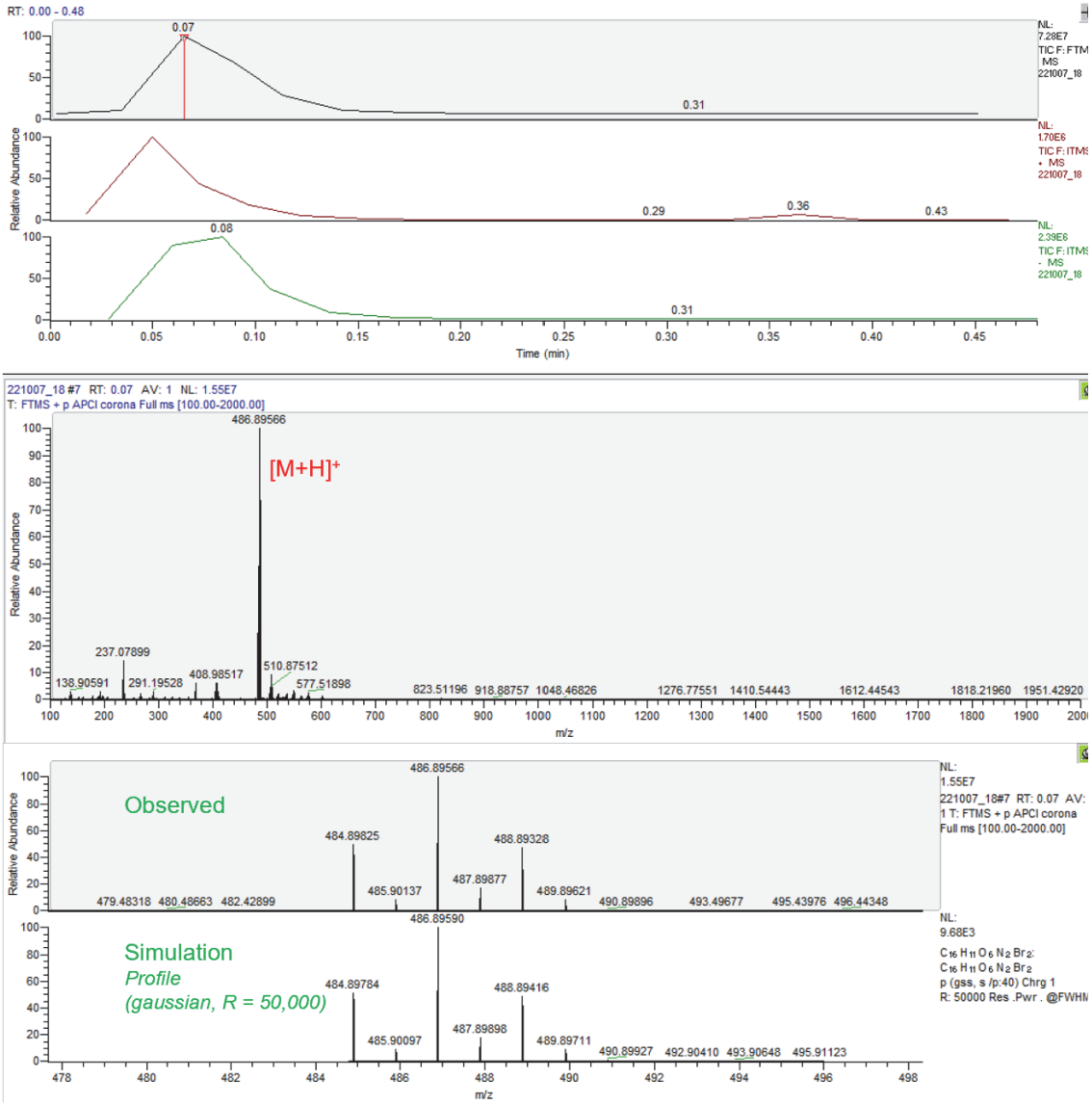


Fig. S20 HRMS spectrum of **4**.

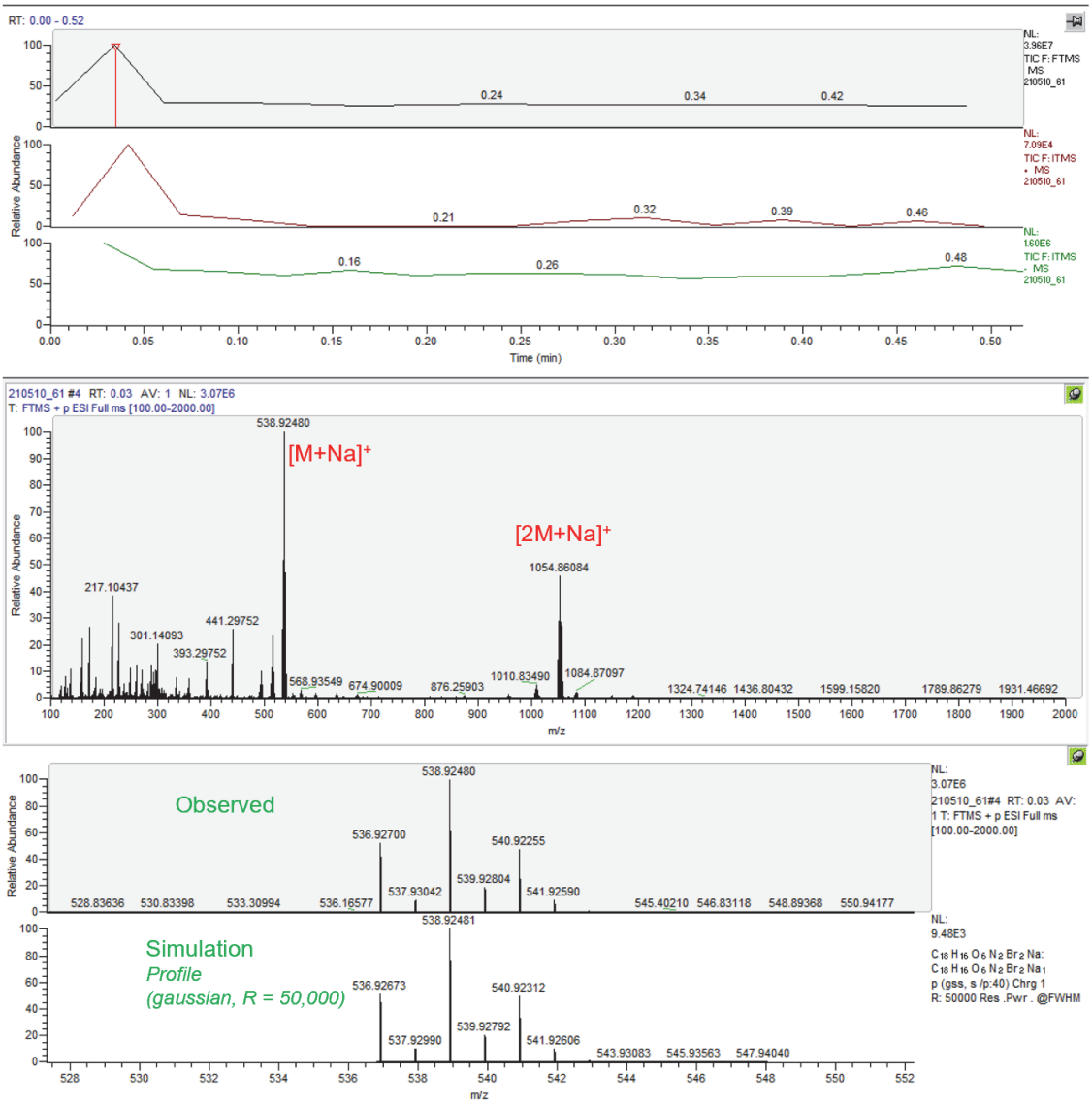


Fig. S21 HRMS spectrum of **5**.

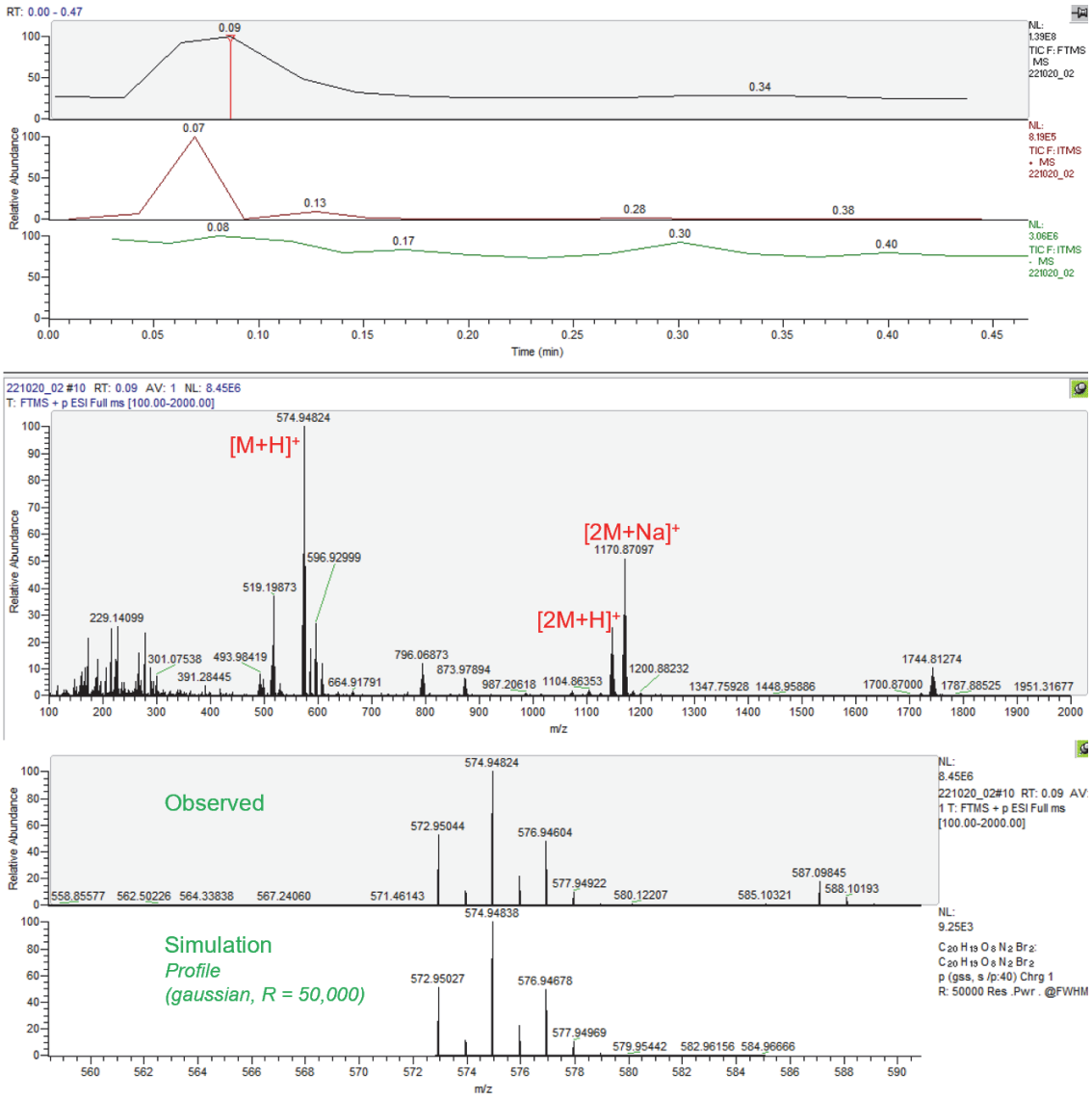


Fig. S22 HRMS spectrum of **6**.

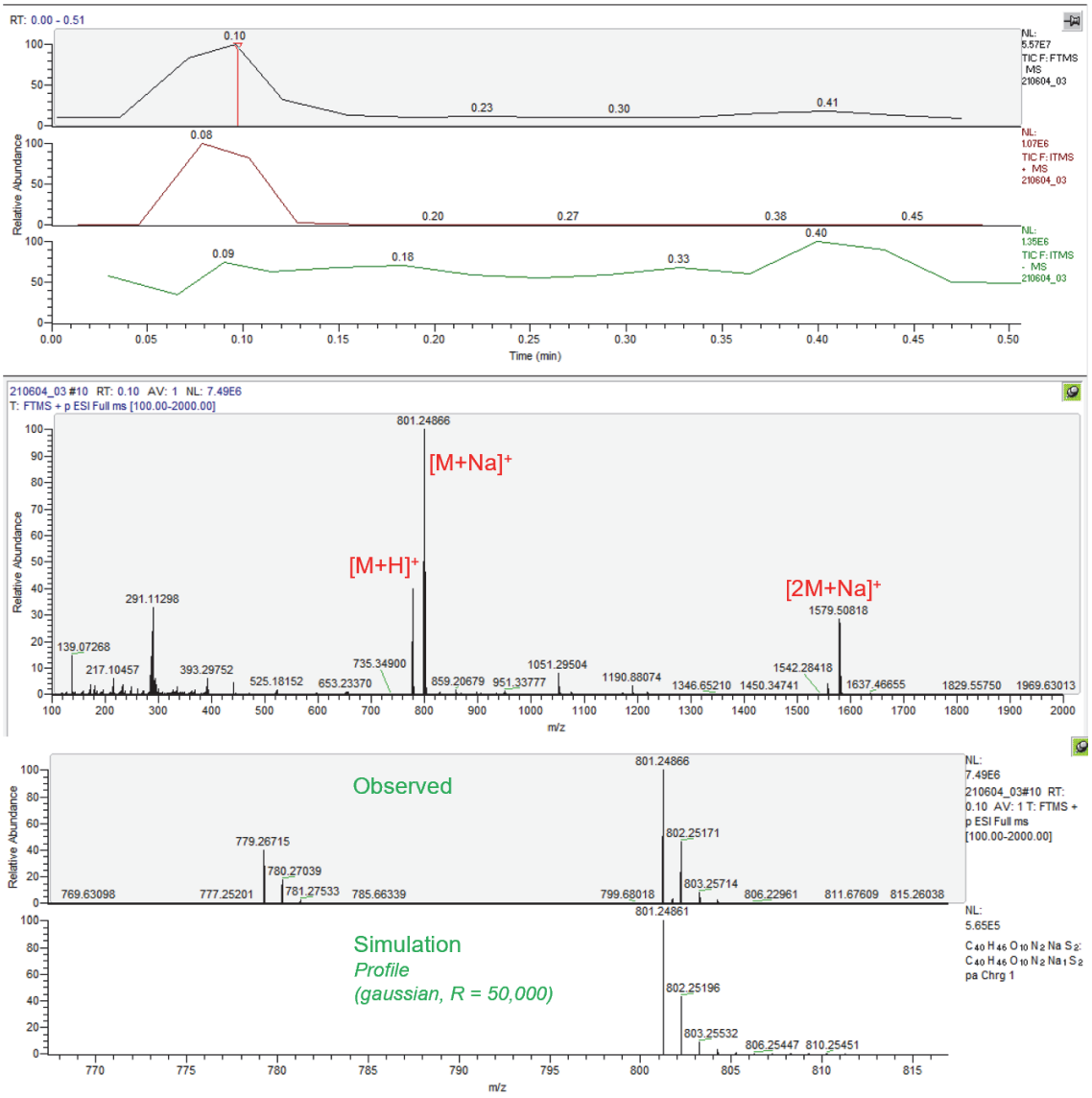


Fig. S23 HRMS spectrum of **7**.

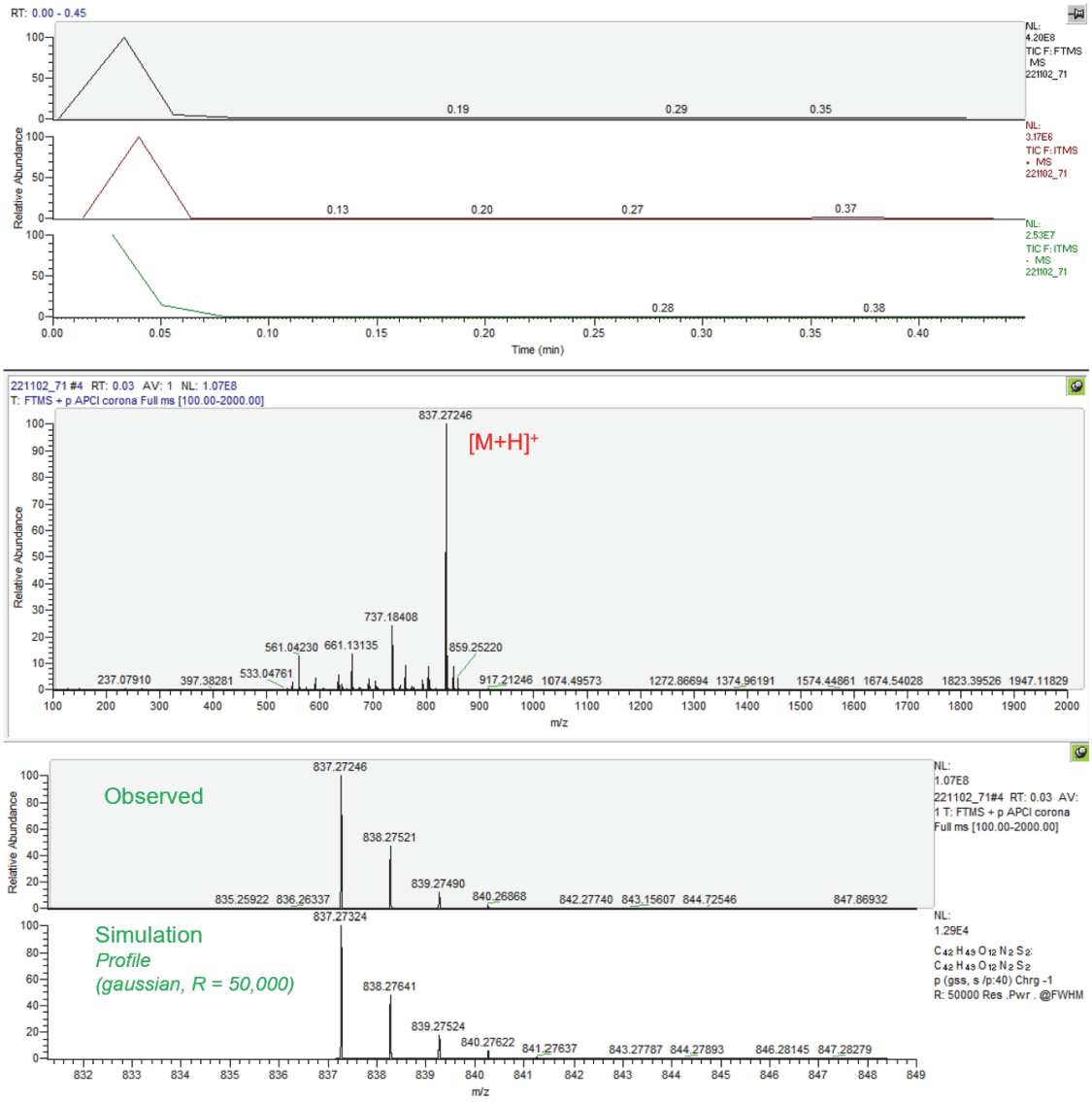


Fig. S24 HRMS spectrum of **8**.

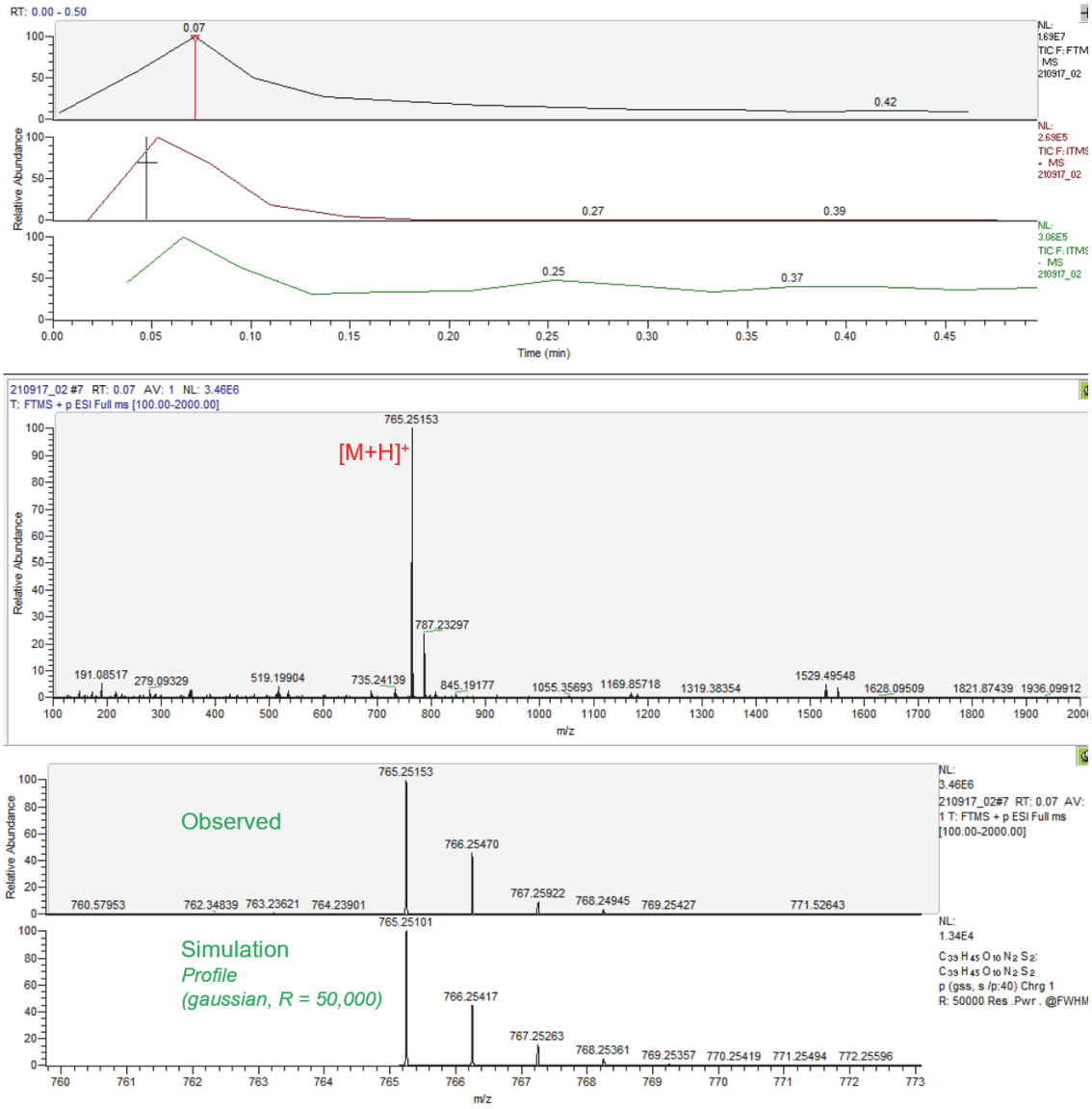


Fig. S25 HRMS spectrum of **9**.

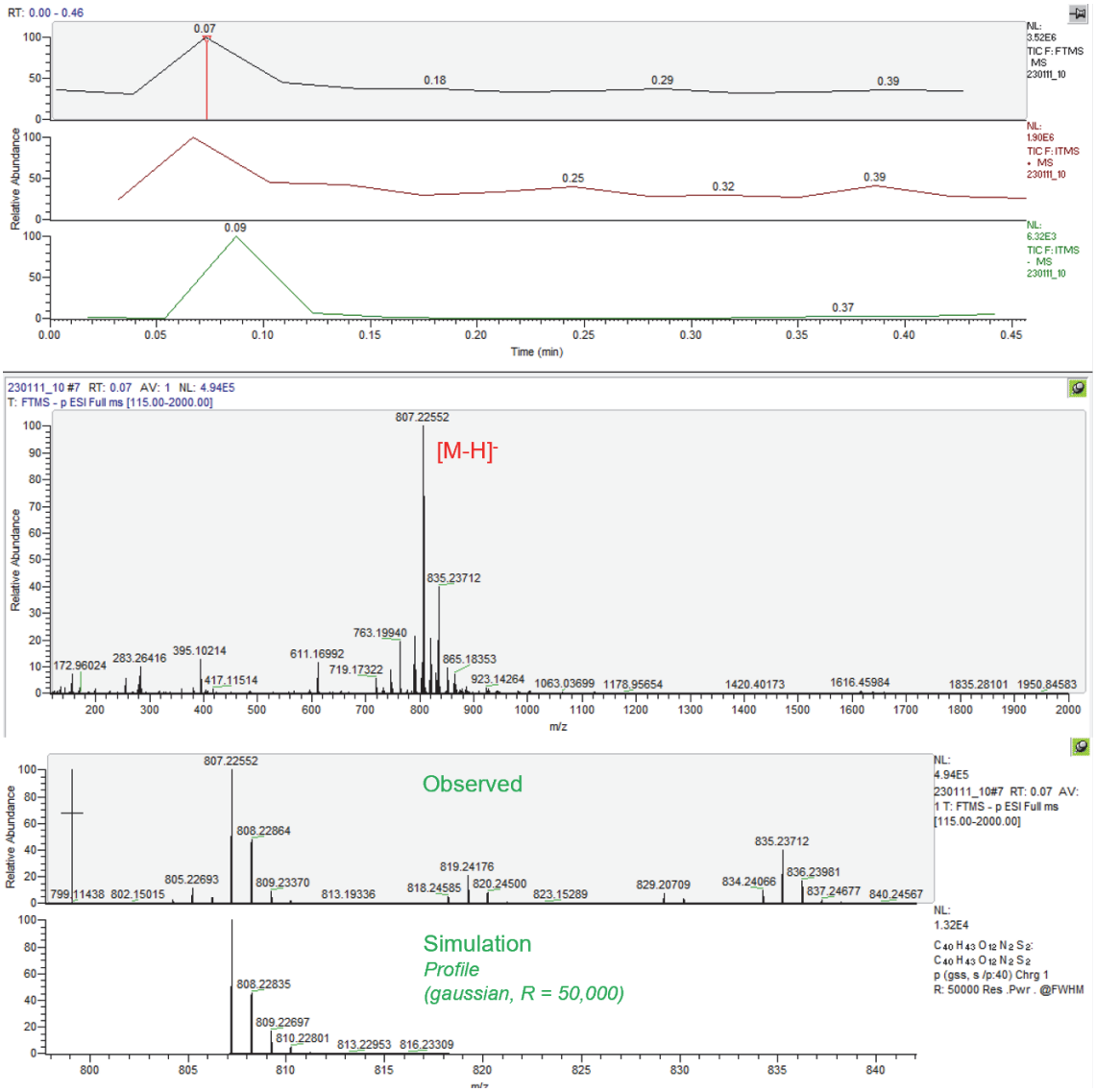


Fig. S26 HRMS spectrum of **10**.

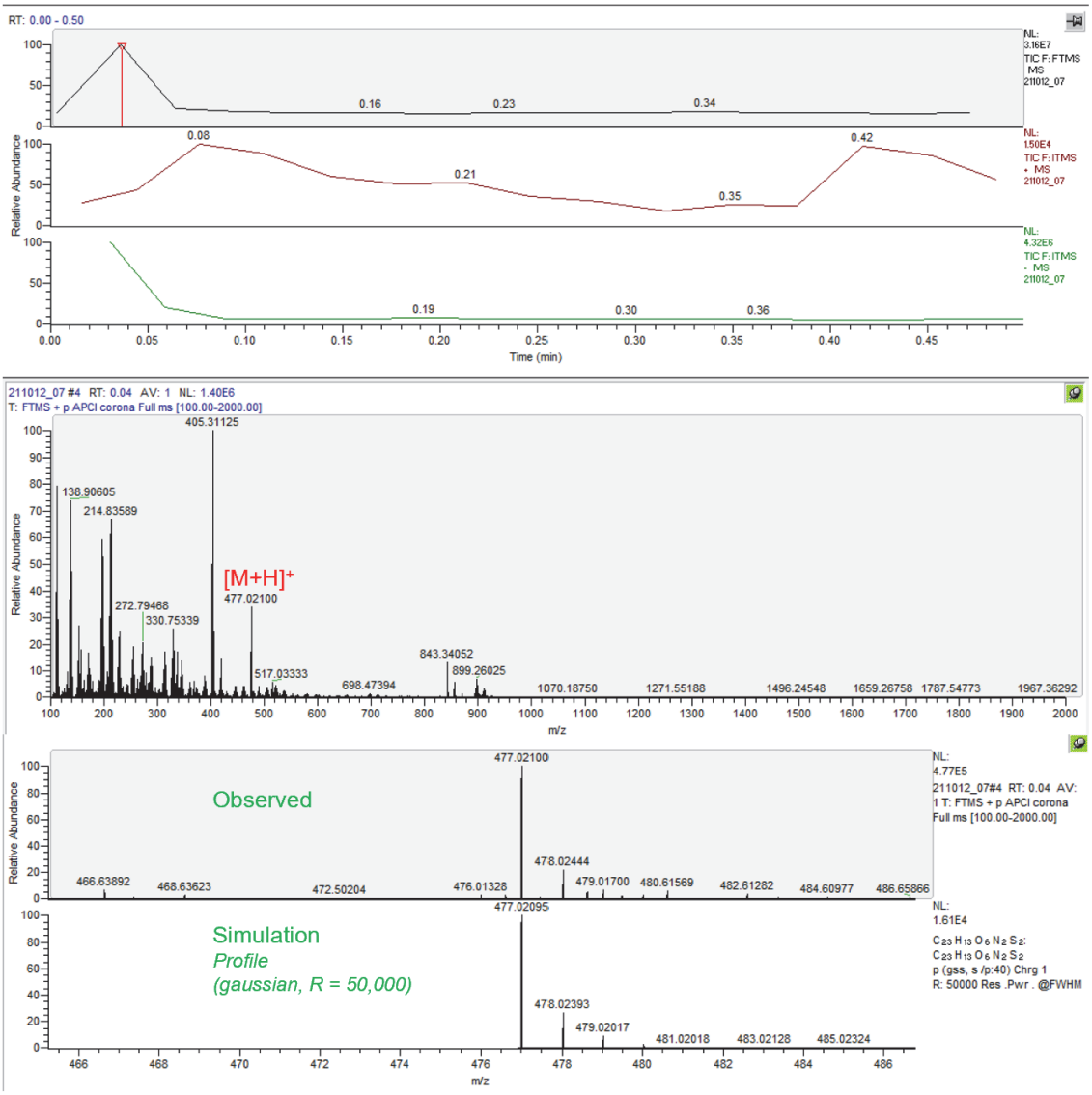


Fig. S27 HRMS spectrum of **KY-1**.

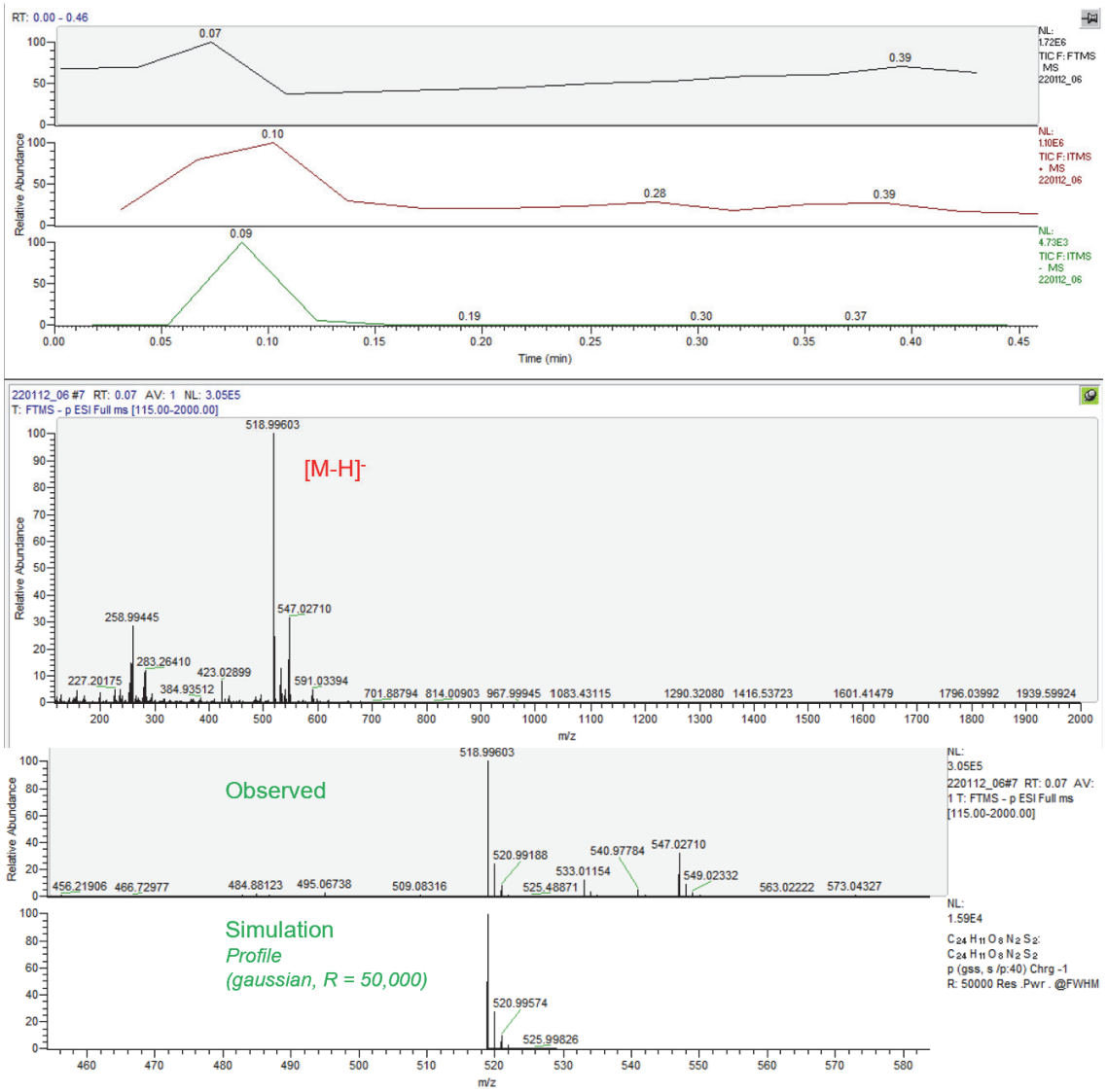


Fig. S28 HRMS spectrum of **KY-2**.

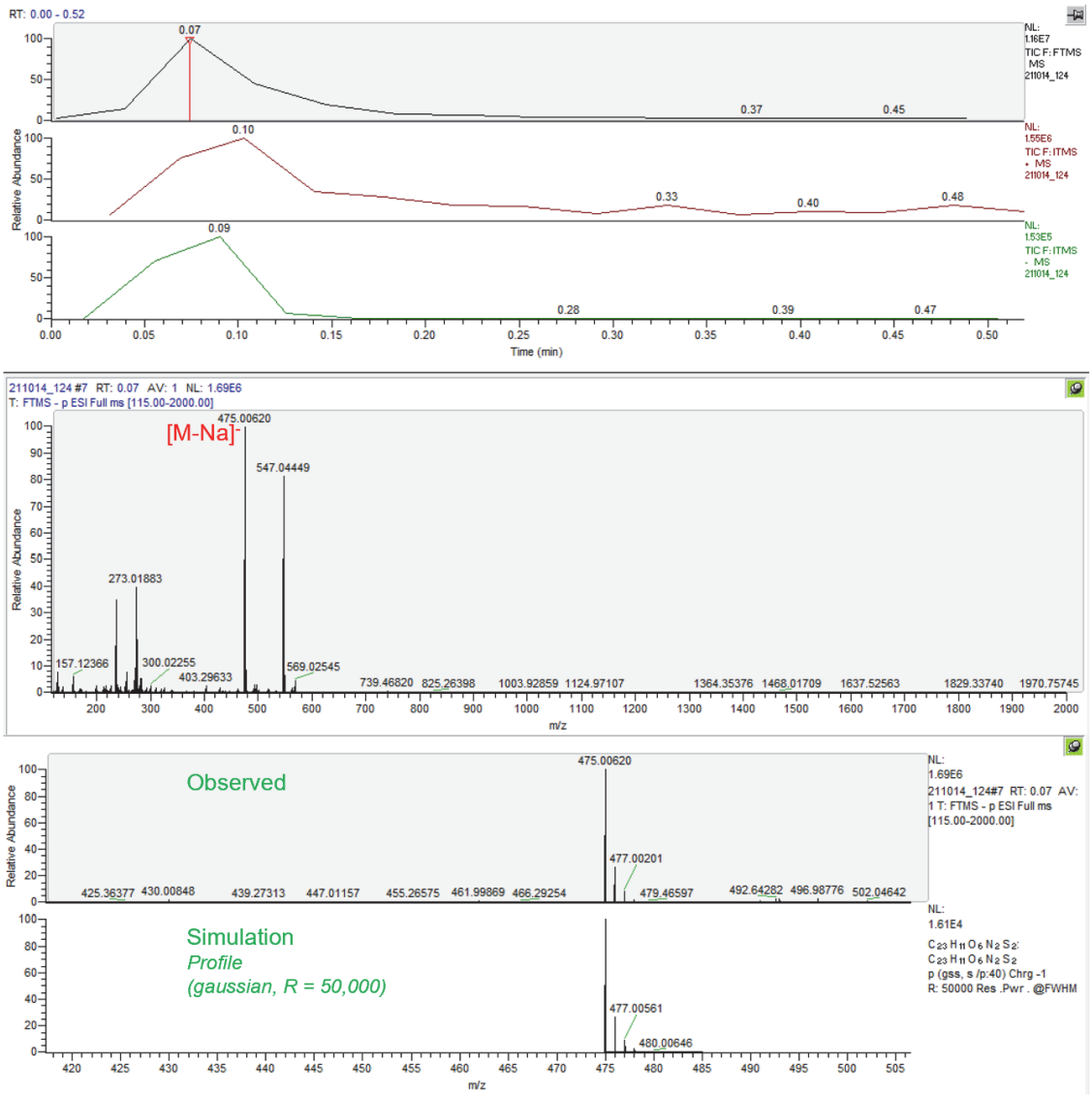


Fig. S29 HRMS spectrum of **KY-1Na**.

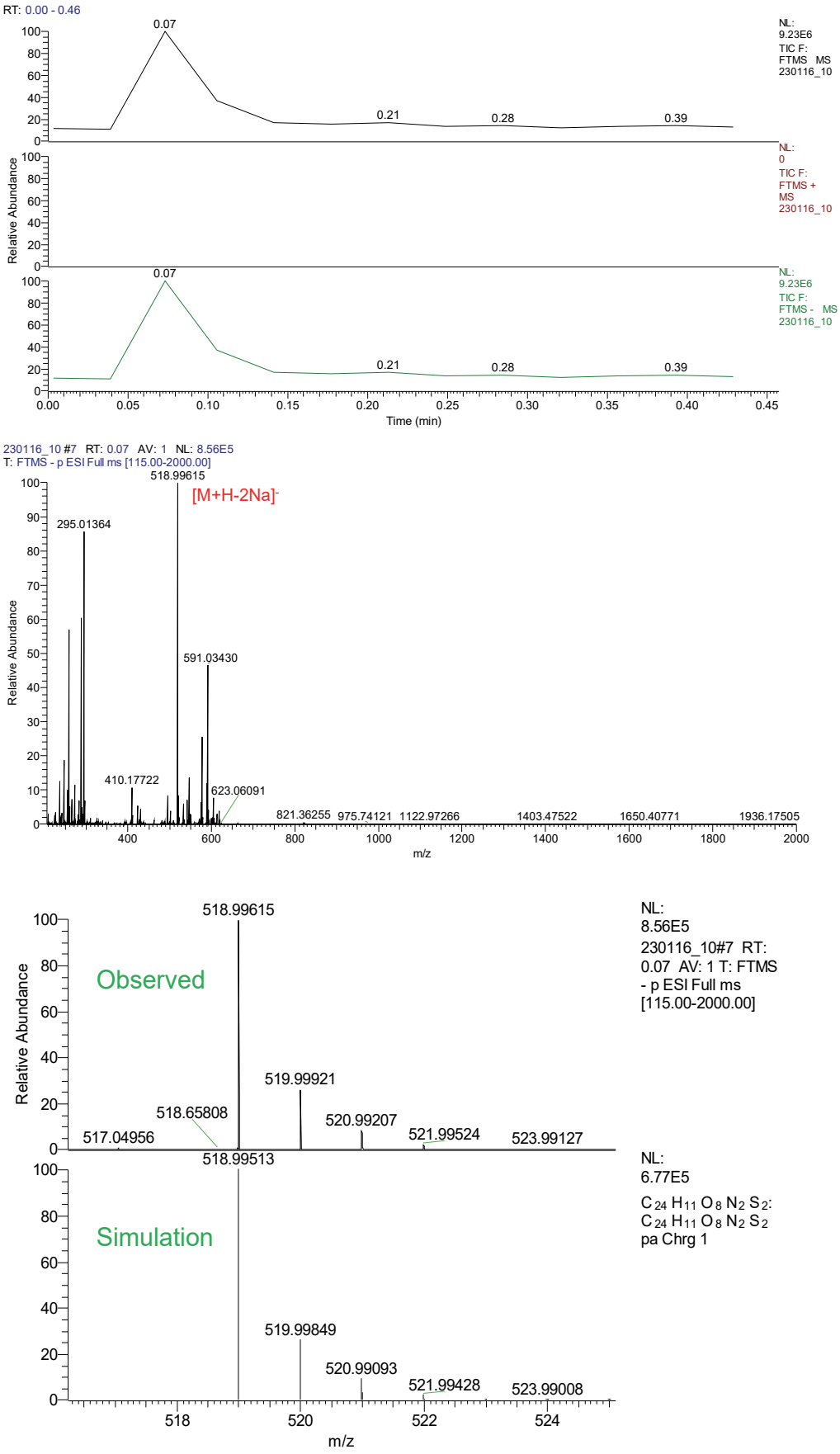


Fig. S30 HRMS spectrum of **KY-2Na**.

IR spectra

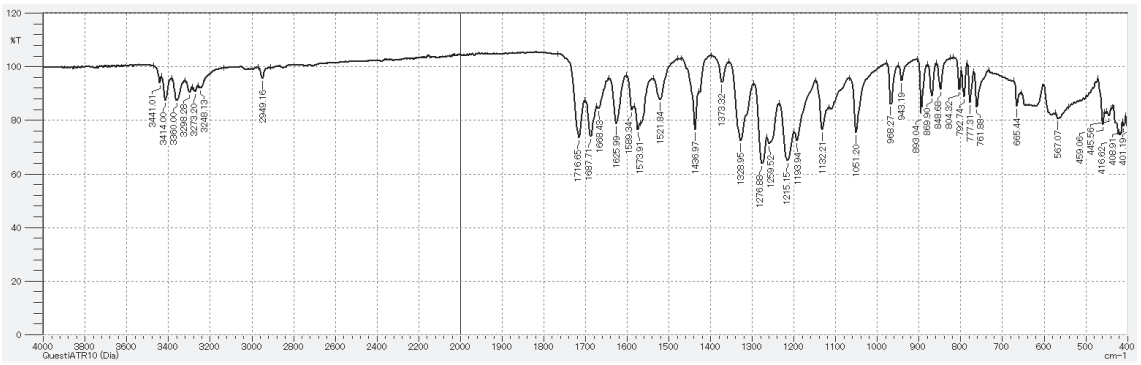


Fig. S31 FT-IR (ATR) spectrum of **1**.

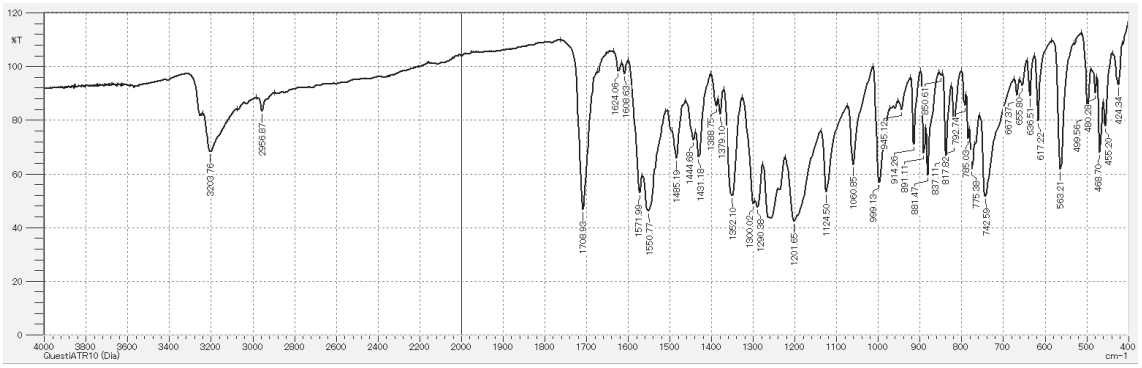


Fig. S32 FT-IR (ATR) spectrum of **3**.

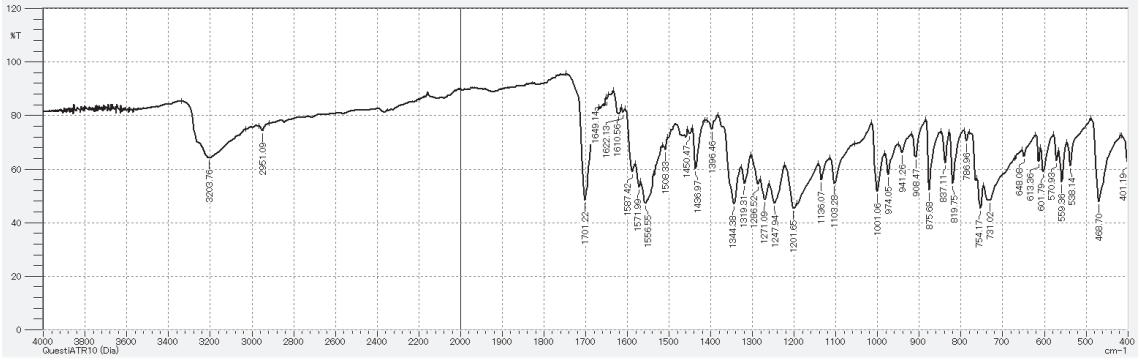


Fig. S33 FT-IR (ATR) spectrum of **4**.

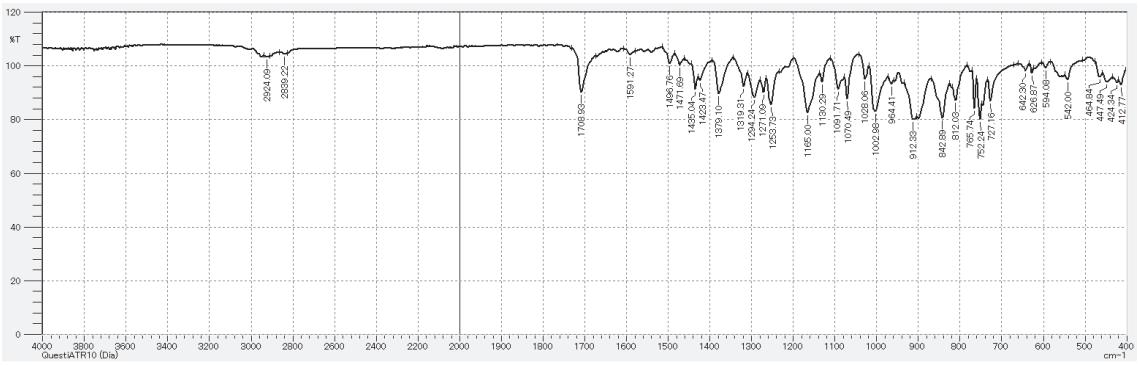


Fig. S34 FT-IR (ATR) spectrum of **5**.

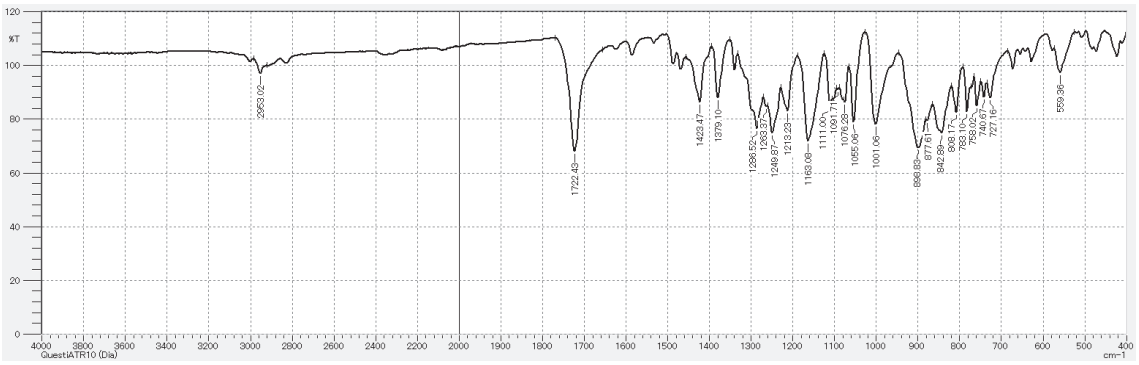


Fig. S35 FT-IR (ATR) spectrum of **6**.

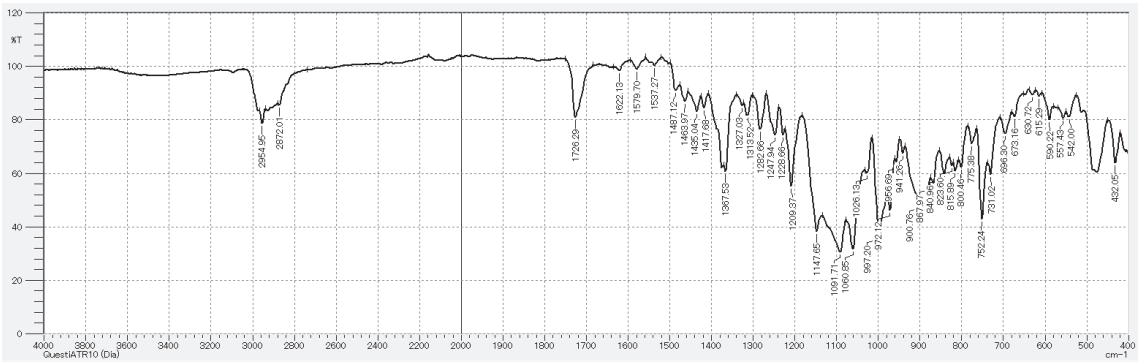


Fig. S36 FT-IR (ATR) spectrum of **7**.

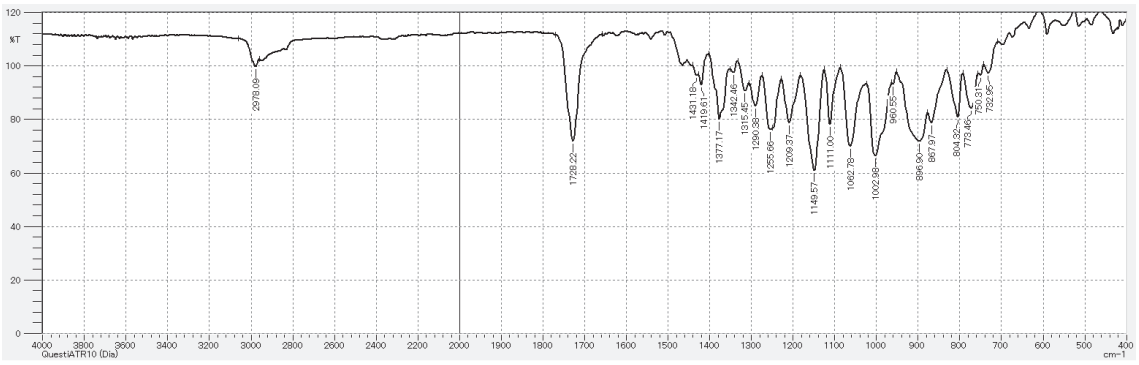


Fig. S37 FT-IR (ATR) spectrum of **8**.

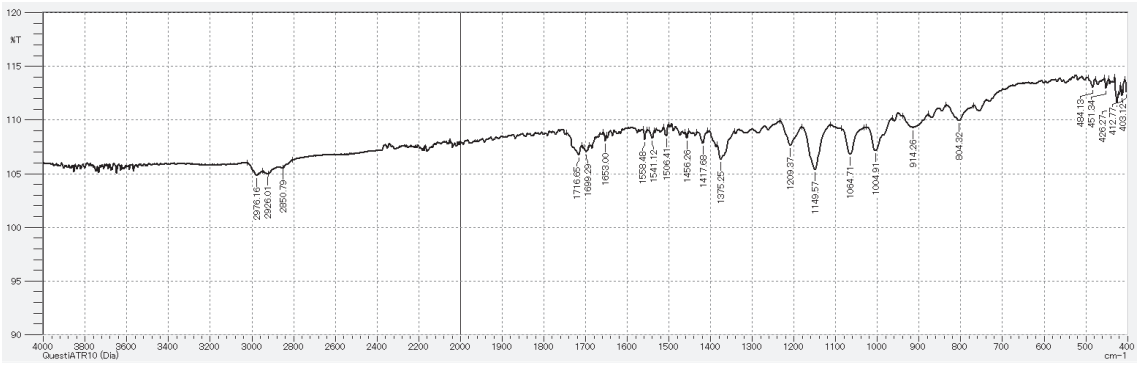


Fig. S38 FT-IR (ATR) spectrum of **9**.

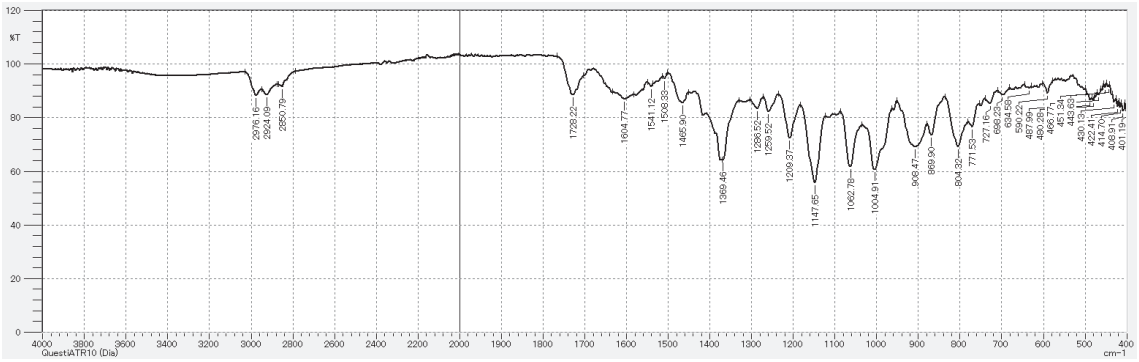


Fig. S39 FT-IR (ATR) spectrum of **10**.

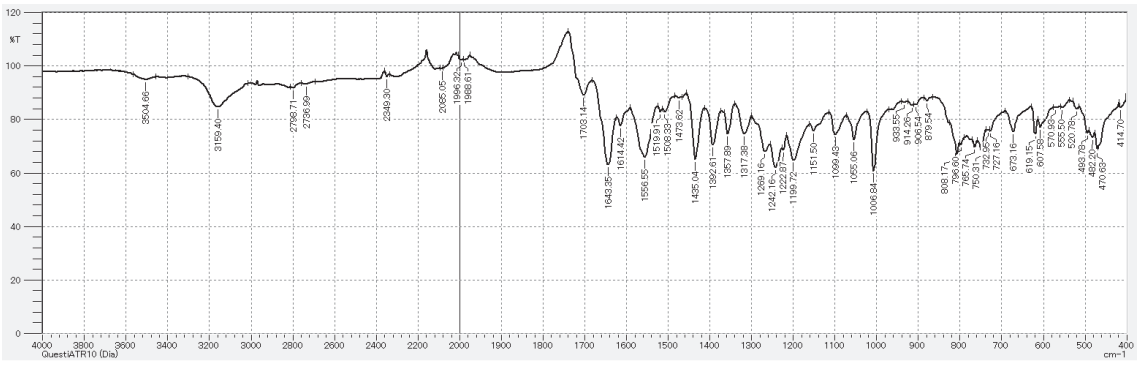


Fig. S40 FT-IR (ATR) spectrum of **KY-1**.

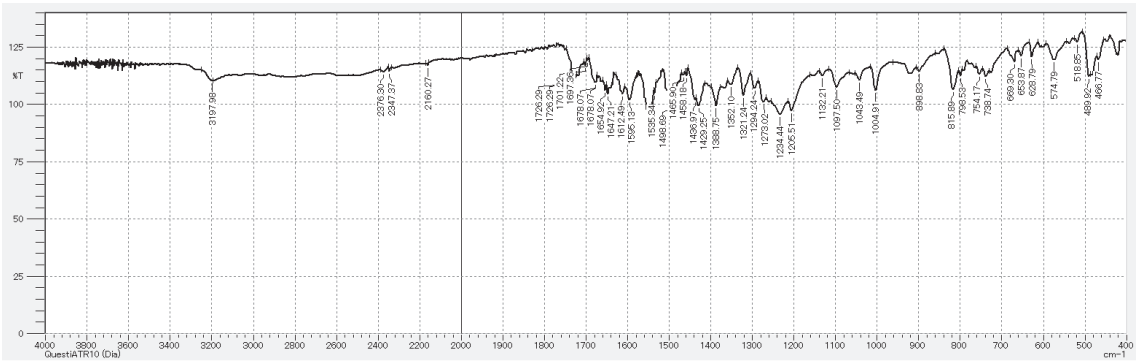


Fig. S41 FT-IR (ATR) spectrum of **KY-2**.

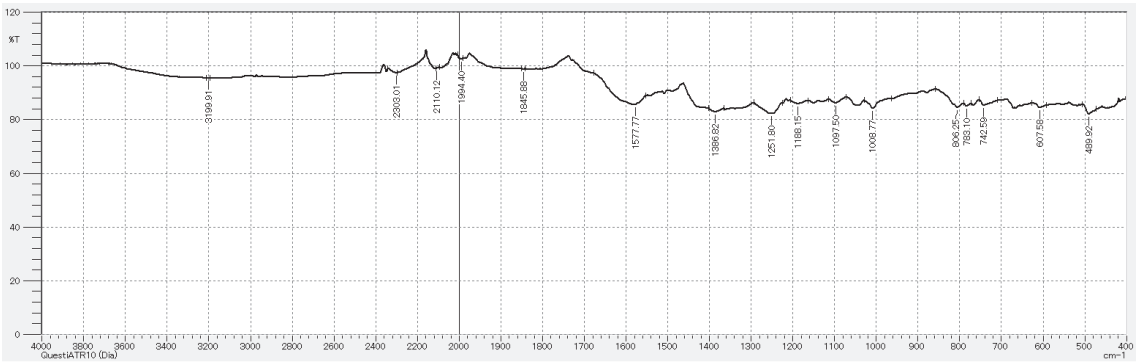


Fig. S42 FT-IR (ATR) spectrum of **KY-1Na**.

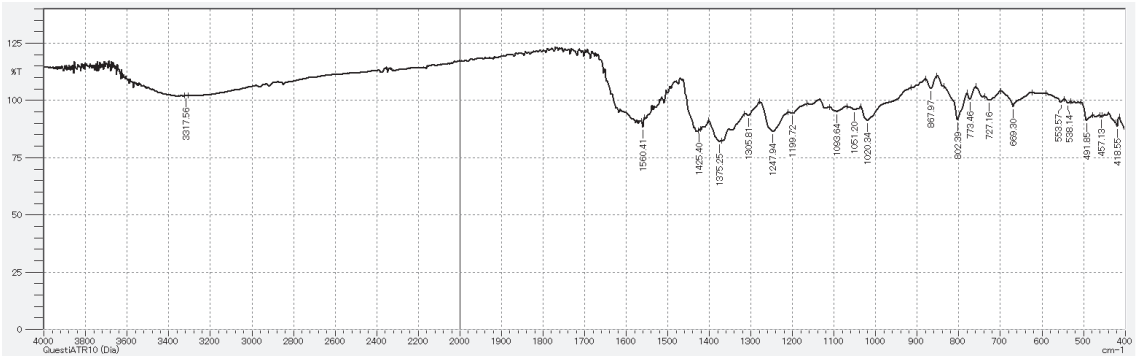
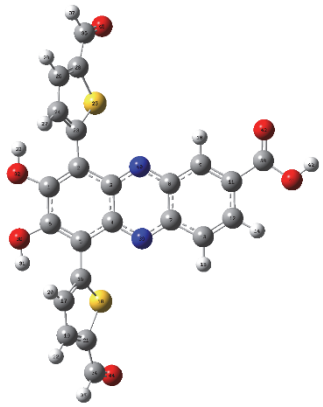


Fig. S43 FT-IR (ATR) spectrum of **KY-2Na**.

Theoretical calculations

Table S1 Geometrical coordinates of the optimized **KY-1** by DFT at the B3LYP/6-311G(d,p)/DMSO-IEFPCM level.



Tag	Symbol	X	Y	Z	Tag	Symbol	X	Y	Z
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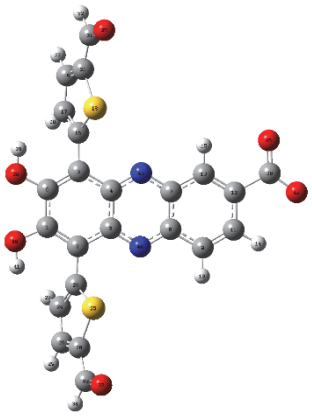
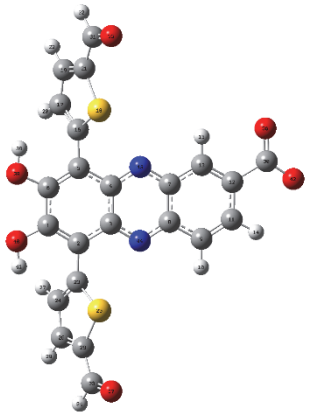
2	C	0.976895	-1.7674	-0.29557	25	S	3.500757	-1.54679	0.935995
3	C	0.510161	-0.40418	-0.27356	26	C	4.433192	-3.09246	-0.9187
4	C	-0.91449	-0.12938	-0.27792	27	H	2.575224	-3.33263	-2.08463
5	C	-1.85167	-1.22112	-0.30481	28	C	4.825965	-2.42356	0.219443
6	C	-1.36845	-2.51342	-0.3031	29	H	5.111988	-3.68351	-1.51969
7	C	-0.47503	2.122418	-0.25472	30	O	-2.14384	-3.61184	-0.2816
8	C	0.932359	1.850184	-0.25156	31	H	-3.07537	-3.34877	-0.22542
9	C	1.848047	2.932966	-0.23933	32	O	0.358847	-4.09679	-0.27479
10	H	2.909688	2.722665	-0.23864	33	H	1.320483	-4.19993	-0.20787
11	C	1.388719	4.22917	-0.2286	34	C	-6.90546	0.104949	0.76317
12	C	-0.01357	4.499215	-0.23116	35	H	-7.81532	-0.16684	0.19495
13	C	-0.91932	3.474629	-0.2446	36	C	6.159339	-2.38851	0.797715
14	H	-0.35069	5.526593	-0.22304	37	H	6.90605	-2.98642	0.241382
15	H	-1.98613	3.66171	-0.24827	38	C	2.395684	5.327822	-0.21567
16	C	-3.30667	-0.99503	-0.3275	39	N	-1.37123	1.119979	-0.27283
17	C	-4.20611	-1.4985	-1.24971	40	N	1.395548	0.58594	-0.26579
18	S	-4.12503	-0.09935	0.922398	41	O	1.831021	6.551115	-0.20595
19	C	-5.54781	-1.16418	-0.95904	42	H	2.54768	7.204211	-0.19826
20	H	-3.90071	-2.06034	-2.12295	43	O	3.595567	5.162559	-0.21407
21	C	-5.67465	-0.41294	0.188325	44	O	-6.97128	0.778197	1.776422
22	H	-6.39214	-1.45403	-1.57088	45	O	6.46627	-1.77132	1.802298
23	C	2.412049	-2.09583	-0.30817					

Table S2 Geometrical coordinates of the optimized **KY-1Na** by DFT at the B3LYP/6-311+G(d,p)/DMSO-IEFPCM level.



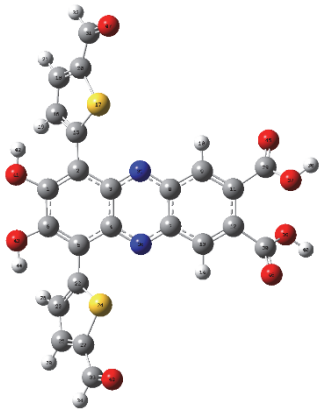
Tag	Symbol	X	Y	Z	Tag	Symbol	X	Y	Z
1	C	1.324116	-2.46013	-0.42434	23	C	3.277859	-0.95894	-0.37063
2	C	1.818323	-1.17524	-0.37389	24	C	4.171151	-1.26847	-1.37723
3	C	0.897828	-0.06998	-0.32452	25	S	4.097156	-0.31329	1.025061
4	C	-0.52531	-0.3257	-0.32106	26	C	5.512414	-0.97681	-1.0382
5	C	-1.00316	-1.68243	-0.37069	27	H	3.862484	-1.66704	-2.33491
6	C	-0.0939	-2.71463	-0.42568	28	C	5.644114	-0.45314	0.230815
7	C	-0.92839	1.933995	-0.2613	29	H	6.354942	-1.13737	-1.69861
8	C	0.48705	2.187006	-0.26687	30	C	-2.34965	5.509544	-0.15255
9	C	0.941772	3.536288	-0.23864	31	C	-6.13263	-2.42655	0.895031
10	H	2.010668	3.717153	-0.24474	32	H	-6.93064	-2.84608	0.255516
11	C	0.039347	4.564971	-0.2029	33	C	6.883248	-0.04853	0.868732
12	C	-1.37001	4.325939	-0.19481	34	H	7.780781	-0.19876	0.241233
13	C	-1.83139	3.032679	-0.22553	35	O	-6.36814	-2.07413	2.040751
14	H	0.375457	5.593611	-0.17981	36	O	-3.57578	5.230221	-0.14661
15	H	-2.89557	2.834176	-0.22154	37	O	6.976275	0.418636	1.993745
16	C	-2.4477	-1.98582	-0.36533	38	O	-0.43542	-4.02097	-0.45863
17	C	-3.19062	-2.5367	-1.38999	39	H	-1.40081	-4.10488	-0.44076
18	S	-3.42334	-1.7246	1.054577	40	O	2.097852	-3.5666	-0.45009
19	C	-4.54714	-2.73833	-1.0454	41	H	3.032236	-3.31052	-0.42086
20	H	-2.77242	-2.76304	-2.36209	42	O	-1.83531	6.658185	-0.12715
21	C	-4.83695	-2.3499	0.245685	43	N	-1.4035	0.675667	-0.29483
22	H	-5.29066	-3.14797	-1.71709	44	N	1.371274	1.175686	-0.30386

Table S3 Geometrical coordinates of the optimized **KY-1Na** by DFT at the B3LYP/6-311+G(d,p)/Water-IEFPCM level.



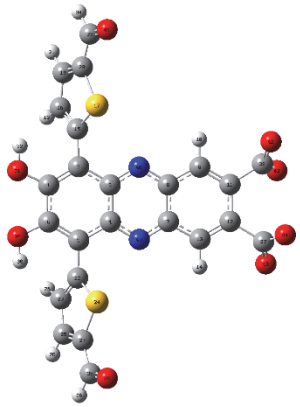
Tag	Symbol	X	Y	Z	Tag	Symbol	X	Y	Z
1	C	1.327674	-2.45807	-0.4257	23	C	3.279216	-0.95396	-0.37185
2	C	1.819953	-1.17251	-0.37484	24	C	4.172251	-1.25729	-1.3804
3	C	0.898002	-0.06867	-0.32479	25	S	4.098278	-0.31359	1.026532
4	C	-0.52494	-0.32652	-0.32159	26	C	5.513317	-0.96497	-1.04086
5	C	-1.00088	-1.6839	-0.37124	27	H	3.863548	-1.65259	-2.33941
6	C	-0.09008	-2.71478	-0.42646	28	C	5.645014	-0.44695	0.23042
7	C	-0.93105	1.93268	-0.2615	29	H	6.355661	-1.12131	-1.70251
8	C	0.483887	2.187739	-0.26599	30	C	-2.35767	5.50577	-0.15341
9	C	0.936596	3.537686	-0.23649	31	C	-6.12912	-2.43084	0.896693
10	H	2.005208	3.720234	-0.24163	32	H	-6.92689	-2.85336	0.25895
11	C	0.032718	4.565127	-0.20092	33	C	6.883696	-0.04349	0.869747
12	C	-1.37626	4.324012	-0.19463	34	H	7.781342	-0.18974	0.241598
13	C	-1.83567	3.030015	-0.22628	35	O	-6.36481	-2.07444	2.041313
14	H	0.367638	5.594131	-0.17677	36	O	-3.58364	5.224945	-0.15264
15	H	-2.89956	2.829796	-0.22369	37	O	6.976174	0.418914	1.996905
16	C	-2.44498	-1.98913	-0.3653	38	O	-0.42932	-4.02154	-0.45915
17	C	-3.18742	-2.54311	-1.38862	39	H	-1.39454	-4.10762	-0.4408
18	S	-3.42052	-1.72615	1.054304	40	O	2.10297	-3.56318	-0.45253
19	C	-4.54365	-2.74566	-1.04331	41	H	3.037131	-3.30603	-0.42474
20	H	-2.76925	-2.77129	-2.3603	42	O	-1.84573	6.655458	-0.12347
21	C	-4.83379	-2.35471	0.246921	43	N	-1.40442	0.673665	-0.29554
22	H	-5.28669	-3.15772	-1.71405	44	N	1.369603	1.177672	-0.30319

Table S4 Geometrical coordinates of the optimized **KY-2** by DFT at the B3LYP/6-311G(d,p)/DMSO-IEFPCM level.



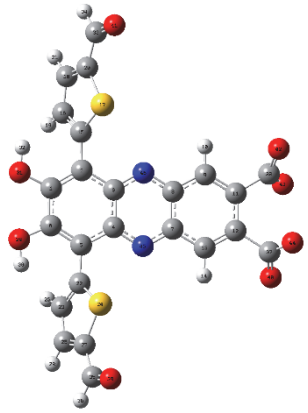
Tag	Symbol	X	Y	Z	Tag	Symbol	X	Y	Z
1	C	-0.71706	-3.22259	-0.33162	25	C	5.094198	-2.65832	-0.93878
2	C	-1.43728	-2.04623	-0.31897	26	H	3.322255	-3.20678	-2.13534
3	C	-0.72401	-0.79723	-0.27893	27	C	5.344711	-1.97135	0.228279
4	C	0.728255	-0.79539	-0.27567	28	H	5.877353	-3.08505	-1.55194
5	C	1.444593	-2.04267	-0.30929	29	C	-1.54206	5.158975	0.053926
6	C	0.727484	-3.22073	-0.32636	30	C	1.530868	5.172374	-0.34031
7	C	0.713935	1.495304	-0.22739	31	C	-6.64592	-1.7161	0.772551
8	C	-0.71545	1.493487	-0.22892	32	H	-7.49107	-2.14293	0.200041
9	C	-1.40679	2.733283	-0.1841	33	C	6.643248	-1.70475	0.825942
10	H	-2.48845	2.72053	-0.1436	34	H	7.494092	-2.12518	0.25714
11	C	-0.72199	3.922123	-0.14525	35	N	-1.40905	0.342122	-0.25941
12	C	0.714147	3.925259	-0.19959	36	N	1.410527	0.345719	-0.2571
13	C	1.402017	2.737714	-0.21741	37	O	-0.9666	6.01388	0.917542
14	H	2.483602	2.729488	-0.26062	38	H	-1.53992	6.792848	0.994615
15	C	-2.90906	-2.09936	-0.33856	39	O	0.955382	6.063199	-1.16688
16	C	-3.69994	-2.74711	-1.26981	40	H	1.526428	6.846619	-1.20696
17	S	-3.87713	-1.39674	0.927617	41	O	-1.26733	-4.44849	-0.32604
18	C	-5.07983	-2.67648	-0.97351	42	H	-2.23219	-4.37017	-0.26932
19	H	-3.29677	-3.22644	-2.15243	43	O	1.281094	-4.44506	-0.31606
20	C	-5.34191	-1.98285	0.187072	44	H	2.245414	-4.3639	-0.25442
21	H	-5.85655	-3.10956	-1.5904	45	O	-2.62813	5.34446	-0.44212
22	C	2.916679	-2.09216	-0.31783	46	O	2.614702	5.338894	0.167146
23	C	3.716896	-2.7315	-1.2467	47	O	-6.83192	-1.08634	1.798578
24	S	3.872076	-1.3951	0.960999	48	O	6.81929	-1.08144	1.857617

Table S5 Geometrical coordinates of the optimized **KY-2Na** by DFT at the B3LYP/6-311+G(d,p)/DMSO-IEFPCM level.



Tag	Symbol	X	Y	Z	Tag	Symbol	X	Y	Z
1	C	-0.69852	-3.15761	-0.41316	24	S	3.842615	-1.4827	1.029942
2	C	-1.41927	-1.9836	-0.36417	25	C	5.11688	-2.41817	-1.01991
3	C	-0.71612	-0.72894	-0.31224	26	H	3.369334	-2.83302	-2.3057
4	C	0.727086	-0.71989	-0.30539	27	C	5.340292	-1.90341	0.240021
5	C	1.445492	-1.96574	-0.35705	28	H	5.917322	-2.73258	-1.67738
6	C	0.739435	-3.14843	-0.41102	29	O	1.314824	-4.37222	-0.43746
7	C	0.709905	1.575027	-0.23814	30	H	2.278773	-4.27771	-0.40761
8	C	-0.72797	1.566016	-0.24845	31	O	-1.25821	-4.38878	-0.43879
9	C	-1.4202	2.80771	-0.21507	32	H	-2.22333	-4.30651	-0.41069
10	H	-2.50414	2.784997	-0.2272	33	C	-6.61021	-1.77844	0.854692
11	C	-0.74611	4.003387	-0.17204	34	H	-7.46179	-2.10566	0.230387
12	C	0.695748	4.012346	-0.15578	35	C	6.63237	-1.70885	0.8705
13	C	1.385832	2.82548	-0.19447	36	H	7.488211	-2.02222	0.244912
14	H	2.47007	2.815864	-0.18354	37	C	1.480863	5.314187	0.003001
15	C	-2.89335	-2.03725	-0.36387	38	C	-1.55091	5.301609	-0.23677
16	C	-3.71129	-2.53063	-1.36179	39	O	6.809379	-1.24708	1.987828
17	S	-3.82358	-1.51889	1.015398	40	O	2.349997	5.571369	-0.86742
18	C	-5.08443	-2.48317	-1.02932	41	O	-6.79364	-1.31141	1.96879
19	H	-3.33086	-2.88668	-2.31039	42	O	-2.42972	5.474686	0.644763
20	C	-5.31529	-1.96233	0.226778	43	O	-1.28466	6.055669	-1.20711
21	H	-5.88041	-2.81124	-1.68552	44	O	1.207579	5.985396	1.030666
22	C	2.920132	-2.00205	-0.35401	45	N	1.407643	0.428019	-0.27428
23	C	3.744778	-2.47902	-1.35435	46	N	-1.41092	0.410538	-0.29299

Table S6 Geometrical coordinates of the optimized **KY-2Na** by DFT at the B3LYP/6-311+G(d,p)/Water-IEFPCM level.



Tag	Symbol	X	Y	Z	Tag	Symbol	X	Y	Z
1	C	-0.69513	-3.15907	-0.41176	24	S	3.844521	-1.4827	1.030988
2	C	-1.41733	-1.98591	-0.36404	25	C	5.119748	-2.40805	-1.02291
3	C	-0.71558	-0.73043	-0.31316	26	H	3.372384	-2.82251	-2.30912
4	C	0.727853	-0.71974	-0.30576	27	C	5.342767	-1.89709	0.238596
5	C	1.447734	-1.96468	-0.35684	28	H	5.92044	-2.71878	-1.6818
6	C	0.743105	-3.14814	-0.40987	29	O	1.319704	-4.37103	-0.43574
7	C	0.707837	1.575155	-0.23884	30	H	2.283661	-4.27598	-0.40712
8	C	-0.72973	1.564587	-0.25074	31	O	-1.25292	-4.39079	-0.43597
9	C	-1.42349	2.805544	-0.21868	32	H	-2.21825	-4.31048	-0.40812
10	H	-2.50738	2.781595	-0.2333	33	C	-6.60809	-1.78302	0.855165
11	C	-0.7506	4.001826	-0.17415	34	H	-7.45972	-2.11292	0.232513
12	C	0.69099	4.012267	-0.15492	35	C	6.634618	-1.70118	0.868993
13	C	1.382521	2.826293	-0.1936	36	H	7.490976	-2.01159	0.242811
14	H	2.46674	2.817834	-0.18071	37	C	1.473217	5.315175	0.007364
15	C	-2.89136	-2.04115	-0.36363	38	C	-1.55528	5.299743	-0.23989
16	C	-3.709	-2.53653	-1.36072	39	O	6.810743	-1.24082	1.987174
17	S	-3.82176	-1.52216	1.015363	40	O	2.345672	5.574606	-0.85915
18	C	-5.08215	-2.49008	-1.02797	41	O	-6.79117	-1.31227	1.967897
19	H	-3.3285	-2.89378	-2.30884	42	O	-2.435	5.473578	0.640722
20	C	-5.31329	-1.96766	0.227381	43	O	-1.28774	6.054371	-1.20965
21	H	-5.87788	-2.81997	-1.68355	44	O	1.194254	5.98646	1.033672
22	C	2.922511	-1.99886	-0.35457	45	N	1.40698	0.428917	-0.27439
23	C	3.747557	-2.4705	-1.35694	46	N	-1.41148	0.408302	-0.29519

Table S7 Vertical excitation energy for S_1 state and T_1–T_4 states and ΔE_{ST} (S_1–T_1) values of **KO-2**, **KY-1**, **KY-2** at the B3LYP/6-311G(d,p)/DMSO-IEFPCM level; **KY-1Na** and **KY-2Na** at the B3LYP/6-311+G(d,p)/DMSO-IEFPCM level; **KY-1Na** and **KY-2Na** at the B3LYP/6-311+G(d,p)/Water-IEFPCM level by TDDFT calculations.

Dye	ΔE_{ST} (S_1–T_1)/eV	S_1/eV	T_1/eV	T_2/eV	T_3/eV	T_4/eV
KO-2[a]	0.9264	2.8456	1.9192	2.4988	2.5745	2.7024
KY-1[a]	0.8518	2.7204	1.8686	2.4581	2.5789	2.6988
KY-2[a]	0.8124	2.7023	1.8899	2.4456	2.613	2.7046
KY-1Na[a]	0.957	2.8787	1.9217	2.538	2.6444	2.7052
KY-2Na[a]	0.8038	2.6814	1.8776	2.3092	2.577	2.6573
KY-1Na[b]	0.9773	2.8995	1.9222	2.5363	2.6459	2.7046
KY-2Na[b]	0.8196	2.7015	1.8819	2.3196	2.582	2.6601

[a] Calculated at DMSO-IEFPCM level. [b] Calculated at Water-IEFPCM level.

Evaluation of $^1O_2$ quantum yield

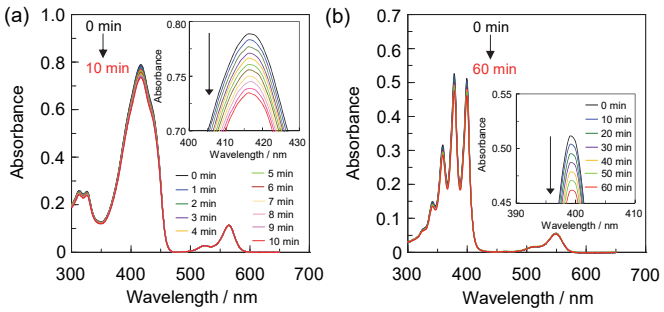


Fig. S44 (a) Photoabsorption spectra for the photooxidation of DPBF (5.0×10^{-5} M) in the presence of **RB** under photoirradiation with 532 nm (300 μW cm^{-2}) in DMSO. Insets are magnifications of maxima in the spectra at around 415 nm. (b) Photoabsorption spectra for the photooxidation of ABDA (5.0×10^{-5} M) in the presence of **RB** under photoirradiation with 532 nm (300 μW cm^{-2}) in D_2O/DMSO (99:1, v/v). Insets are magnifications of maxima in the spectra at around 400 nm.

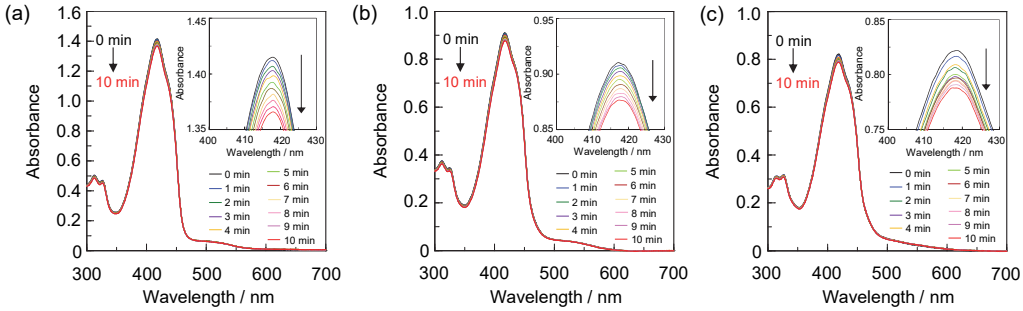


Fig. S45 Photoabsorption spectra for the photooxidation of DPBF (5.0×10^{-5} M) in the presence of (a) **KO-2**, (b) **KY-1**, and (c) **KY-2** under photoirradiation with 532 nm (300 μW cm^{-2}) in DMSO. Insets are magnifications of maxima in the spectra at around 415 nm.

Photodynamic activity against cancer cell lines

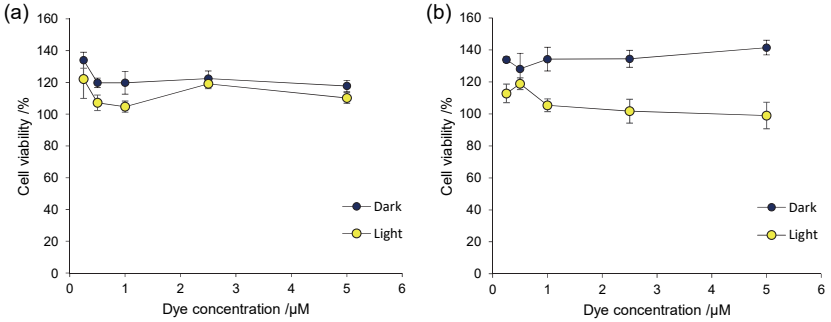


Fig. S46 Photoirradiation-induced anticancer effects on Colon26 cells. Colon26 cells were coincubated with (a) **KY-1Na** or (b) **KY-2Na** for 24 h. Subsequently, the cells were photoirradiated (> 510 nm, 9 mW/cm^2) for 30 min. After an additional 24 h, the cell viability was measured with the WST-8 assay (N = 3). The data were presented as mean ± SD.

Evaluation of cellular uptake of KY-2Na

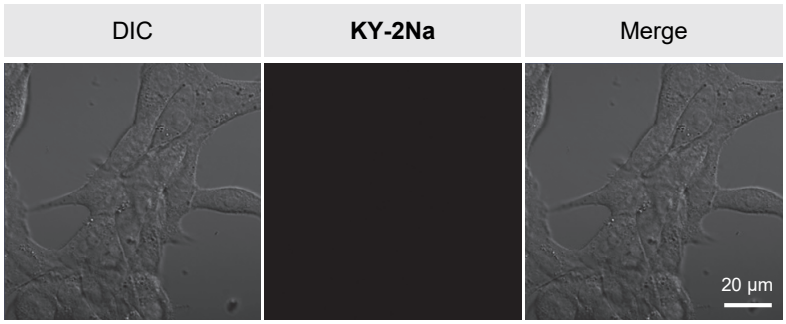


Fig. S47 Observation of cellular uptake of **KY-2Na** in the Colon26 cells by confocal laser scanning microscopy (λ^{ex} = 488 nm) and differential interference contrast (DIC). After 24 h of incubation with **KY-2Na**. Scale bar = 20 μm.