Supplementary Material (ESI) for Chemical Communications
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Supporting Information

Water Soluble Distyryl-Boradiazaindacenes as Efficient Photosensitizers for Photodynamic Therapy

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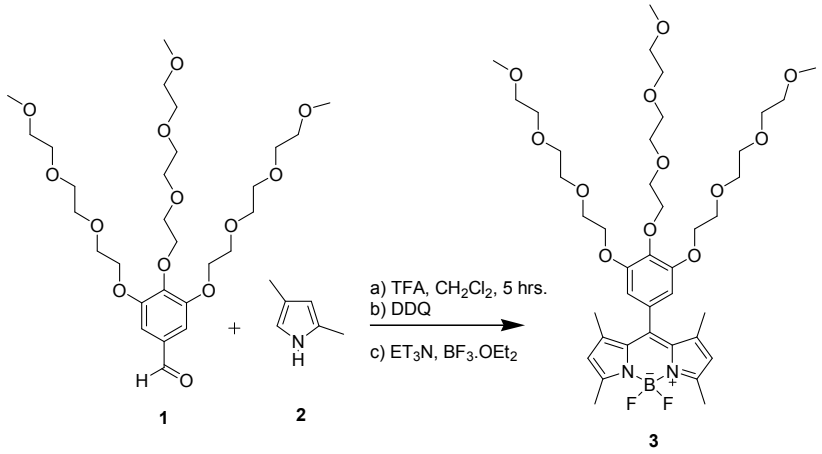
EXPERIMENTAL PROCEDURES

General

All chemicals and solvents purchased from Aldrich were used without further purification. ^{1}H NMR and ^{13}C NMR spectra were recorded using a Bruker DPX-400 in $CDCl_3$ or DMSO-d_6 with TMS as internal reference. Absorption spectrometry was performed using a Varian spectrophotometer. Steady state fluorescence measurements were conducted using a Varian Eclipse spectrofluorometer. Column chromatography of all products was performed using Merck Silica Gel 60 (particle size: 0.040–0.063 mm, 230–400 mesh ASTM). Reactions were monitored by thin layer chromatography using fluorescent coated aluminum sheets. Solvents used for spectroscopy experiments were spectrophotometric grade. Mass spectrometry measurements were done at the Ohio State University Mass Spectrometry and Proteomics Facility, Ohio, U.S.A., or Hacettepe University, Mass Spectrometry Facility, Ankara, Turkey.

Synthesis:

4,4-difluoro-8-[3,4,5-tris(2-(2-(2-methoxyethoxy)ethoxy)ethoxy)]benzaldehyde-1,3,5,7-tetramethyl -4-bora-3a,4a-diaza-s-indacene (3).

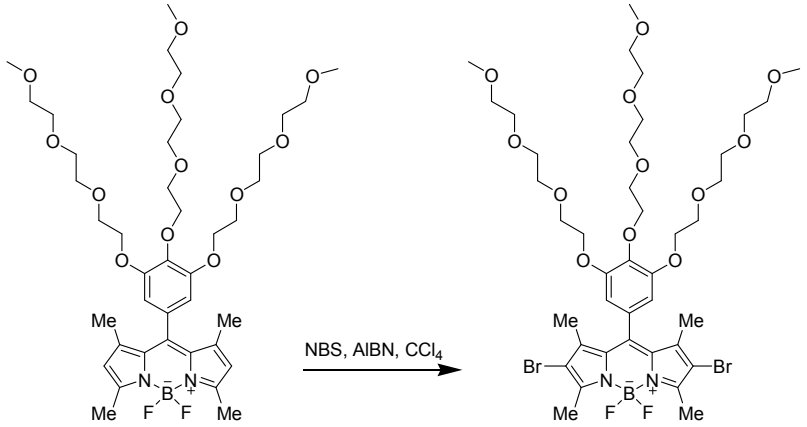


3,4,5-tris(2-(2-(2-methoxyethoxy)ethoxy)ethoxy)benzaldehyde (**1**) (0.34 mmol, 200 mg) and 2,4-dimethylpyrrole (2) (0.68 mmol, 8 4mg) were dissolved in CH_2Cl_2 (250 mL) purged with argon in a 100 mL flask. 1 drop of TFA was added and the mixture was stirred at room temperature for 3 hrs. When TLC showed consumption of the aldehyde was complete, a solution of 166 mg (0.68 mmol) of DDQ (Tetrachloro-1,4-benzoquinone) in CH_2Cl_2 was added. After 3 h, Et_3N (3 ml) and $BF_3.OEt_2$ (3 ml) were added. Immediately after the addition of $BF_3.OEt_2$ bright green fluorescence was observed. Crude product washed three times with water, dried over Na_2SO_4 and concentrated in vacuo. Then crude product purified by silica gel column chromatography using $CHCl_3/CH_3OH$ (99/1, v/v). The orange fraction which has bright green fluorescence was collected. Orange solid (0.147 mmol, 120 mg, 43 %).

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.48 (s, 2H), 5.91 (s, 2H), 4.15 (t, *J*= 5. 0 Hz, 2H), 4.06 (t, *J*= 5. 0 Hz, 4H), 3.76 (t, *J*= 4. 9 Hz, 4H), 3.42- 3.70 (m, 26H), 3.30 (s, 3H), 3.27 (s, 6H), 2.48 (s, 6H), 1.46 (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.6, 153.7, 143.0, 141.2, 139.2, 131.3, 129.9, 121.1, 107.8, 72.7, 72.0, 71.9, 70.9, 70.7, 70.6, 70.5, 69.8, 69.3, 59.0, 14.5, 14.3.

ESI-HRMS calcd for M+Na 833.4191, found 833.4171 , Δ= 2.4 ppm

2,6-dibromo-4,4-difluoro-8-[3,4,5-tris(2-(2-(2-methoxyethoxy)ethoxy)ethoxy)]-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacene (4).



3 **4**

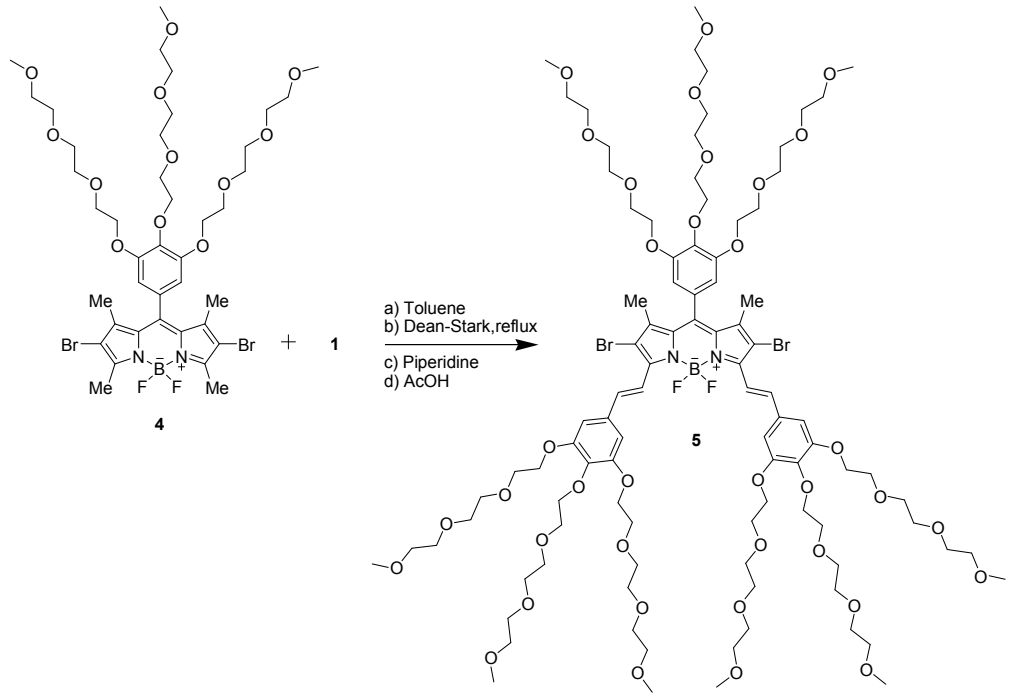
Boradiazaindacene dye **3** (0.123 mmol, 100 mg), α,α'-Azoisobutyronitrile (AIBN) (0.0123 mmol, 2.0 mg) and N-Bromosuccinimide (NBS) (0.246 mmol, 43.78 mg) were refluxed in CCl_4 (20 ml). After 30 min, crude product concentrated under vacuo, then purified by sillica gel column chromatography using $CHCl_3$. The red colored fraction was collected then the solvent was removed under reduced pressure to yield the desired compound **4** (0.0984 mmol, 95.28 mg, 81%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.30 (s, 2H), 4.00 (t, *J*= 4.9 Hz, 2H), 3.90 (t, *J*= 4.9 Hz, 4H), 3.24-3.64 (m, 30H), 3.14 (s, 3H), 3.12 (s, 6H), 2.34 (s, 6H), 1.28 (s, 6H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 154.0, 141.6, 140.5, 139.6, 130.3, 129.1, 111.7, 107.5, 107.3, 72.8, 72.5, 72.0, 71.9, 71.8, 71.2, 70.8, 70.7, 70.6, 70.5, 70.5, 70.4, 69.7, 69.3, 61.7, 58.9, 58.7, 13.9, 13.6;

ESI-HRMS calcd for M+Na 991.2385, found 991.2379 , Δ= 0.6 ppm

Boradiazaindacene dye 5. (Sensitizer 3 in the article)



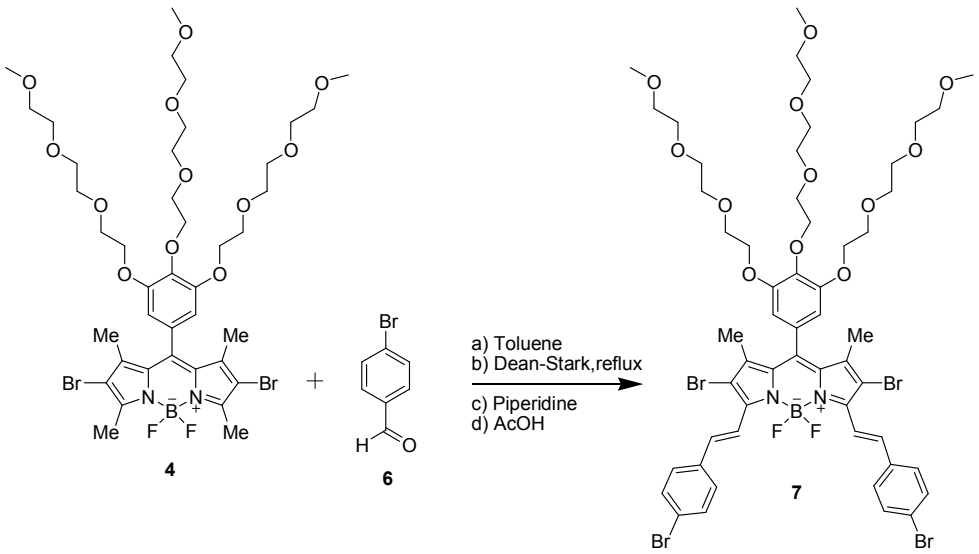
Compound **4** (0.093 mmol, 90 mg) and 1(0.186 mmol, 110.2 mg) were refluxed in a mixture of toluene (20 ml), glacial acetic acid (1.5 mL), and piperidine (2 mL). Any water formed during the reaction, was removed azeotropically by heating overnight in a Dean-Stark apparatus. Solvents were removed under reduced pressure, and the crude product was then purified by silica gel column chromatography using $CHCl_3/CH_3OH$ (95/5, v/v). The green colored fraction was collected then the solvent was removed under reduced pressure to yield compound **5** (0.0186 mmol, 39.5 mg, 21%).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.91- 7.86 (d, *J*= 16 Hz, 2H) 7.44- 7.39 (d, *J*= 16 Hz, 2H), 6.78 (s, 4H), 6.50 (s, 2H), 4.22- 3.22 (m, 135H), 1.50 (s, 6H);

^{13}C NMR (100 MHz, $CDCl_3$) δ 154.0, 153.0, 148.5, 141.1, 140.4, 139.7, 139.5, 139.0, 132.3, 132.2 129.5, 117.2, 110.7, 108.0, 107.9, 72.8, 72.5, 72.0, 71.9, 70.9, 70.8, 70.7, 70.6, 70.5, 69.7, 69.4, 69.2, 59.0, 53.4, 13.8.

MALDI-MS, M^+ 2117.5 (calcd 2117.8); $(M-F)^+$ 2098.5 (calcd 2098.8).

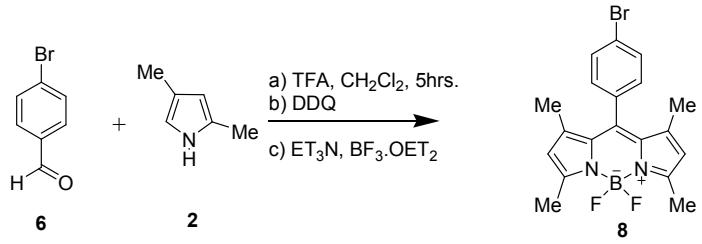
Boradiazainadecene dye 7 (Sensitizer 1 in the article)



Compound **4** (0.103 mmol, 100 mg) and 4-bromobenzaldehyde **6**, (0.206 mmol, 38.0 mg) were refluxed in a mixture of toluene (20 ml), glacial acetic acid (1.5 mL), and piperidine (2.0 mL). Any water formed during the reaction, was removed azeotropically by heating overnight in a Dean-Stark apparatus. Solvents were removed under reduced pressure, and the crude product was then purified by silica gel column chromatography using $CHCl_3$/Hexane (75/25, v/v). The green colored fraction was collected then the solvent was removed under reduced pressure to yield compound **7** (0.021 mmol, 27.0 mg, 20%).

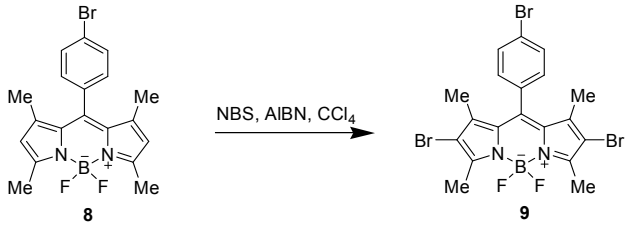
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.04- 7.95 (d, *J*= 16Hz, 2H), 7.57- 7.66 (d, *J*= 16 Hz, 2H), 7.44 (q, *J*= 8 Hz, 8H), 6.49 (s, 2H), 4.20 (t, *J*= 4.9 Hz, 2H), 4.08 (t, *J*= 4.9 Hz, 4H), 3.82- 3.41 (m, 30H), 3.32 (s, 3H), 3.27 (s, 6H), 1.50 (s, 6H);
^{13}C NMR (100 MHz, $CDCl_3$) δ 154.1, 148.3, 141.6, 139.8, 139.7, 138.0, 135.7, 132.3, 132.0, 129.3, 129.1, 123.5, 118.7, 110.6, 107.9, 72.8, 72.0, 71.9, 70.9, 70.7, 70.6, 70.5, 69.8, 69.4, 59.0, 13.8.
MALDI-MS, M^+ 1302.1 (calcd 1302.1); $(M\text{-}F)^+$ 1283.1 (calcd 1283.1).

4,4-difluoro-8-(4-bromophenyl)-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacene



Compound **8** has been reported in the literature. Thus, 4-Bromobenzaldeyhde (**6**) (1.08 mmol, 200 mg) and 2,4-dimethylpyrrole (**2**) (2.16 mmol, 205.0 mg) were dissolved in CH_2Cl_2 (250 mL) purged with argon in a 100 mL flask under argon atmosphere. 1 drop of TFA was added and the mixture was stirred at room temperature for 3 hrs. When TLC showed consumption of the aldehyde was complete, a solution of DDQ (1.08 mmol, 177.5 mg) in CH_2Cl_2 (20 mL) was added. After 3 h, Et_3N (3 ml) and $BF_3.OEt_2$ (3 ml) were added. Immediately after the addition of $BF_3.OEt_2$ bright green fluorescence was observed. Crude product washed three times with water, dried over Na_2SO_4 and concentrated in vacuo. Then crude product purified by silica gel column chromatography using $CHCl_3$. The orange fraction which has bright green fluorescence was collected. Orange solid (0.46 mmol, 187 mg, 45%). Used without further purification in the next step.

2,6-dibromo-4,4-difluoro-8-(4-bromophenyl)-1,3,5,7-tetramethyl-4-bora-3a,4a-diaza-s-indacene



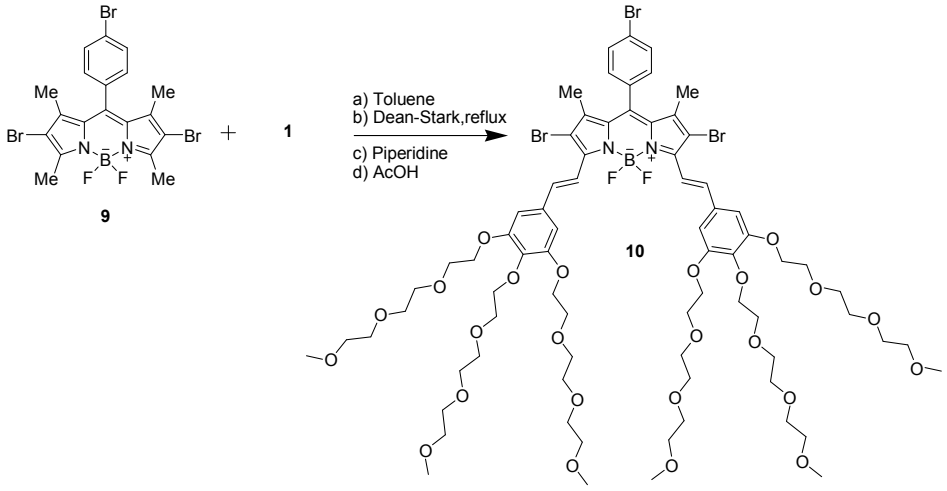
Compound **8** (0.37 mmol, 150 mg), NBS (0.74 mmol, 132.0 mg), AIBN (0.037 mmol, 6.0 mg) were refluxed in CCl_4 (20 ml). After 30 min, crude product concentrated under vacuum, then purified by sillica gel column chromatography using $CHCl_3$. The red colored fraction was collected then the solvent was removed under reduced pressure to yield the desired compound **9** (0.29 mmol, 162 mg, 78%) .

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.60 (d, *J*= 8.2 Hz, 2H), 7.08 (d, *J*= 8.2 Hz, 2H), 2.53 (s, 6H), 1.34 (s, 6H)

^{13}C NMR (100 MHz, $CDCl_3$) δ 154.5, 140.4, 133.3, 132.8, 130.2, 129.7, 124.0, 112.0, 14.0, 13.7.

ESI-HRMS calcd for M+Na 582.8806, found 582.8806 , Δ= 0 ppm

Boradiazainadecene dye 10 (Sensitizer 2 in the article)



Compound **9** (0.178 mmol, 100.0 mg) and 1 (0.356 mmol, 211.0 mg) were refluxed in a mixture of toluene (20 ml), glacial acetic acid (1.5 mL), and piperidine (2.0 mL). Any water formed during the reaction, was removed azeotropically by heating overnight in a Dean-Stark apparatus. Solvents were removed under reduced pressure, and the crude product was then purified by silica gel column chromatography using $CHCl_3/CH_3OH$ (97/3, v/v). The green colored fraction was collected then the solvent was removed under reduced pressure to yield compound **10** (0.043 mmol, 74 mg, 24%).

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^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92-7.85 (d, *J*= 16.5 Hz, 2H), 7.65-7.60 (d, *J*= 8.3 Hz, 2H), 7.47-7.38 (d, *J*= 16.5 Hz, 2H), 7.15-7.11 (d, *J*= 8.3 Hz, 2H), 6.77 (s, 4H), 4.19-4.11 (m, 12H), 3.82-3.41(m, 60 H), 3.31 (s, 6H), 3.28 (s, 12 H), 1.41 (s, 6H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 152.9, 148.8, 140.5, 139.8, 132.7, 132.2, 132.0, 130.5, 130.2, 129.0, 128.4, 125.5, 124.0, 117.2, 111.0, 107.9, 77.3, 77.0, 76.7, 72.5, 71.9, 70.8, 70.7, 70.5, 69.7, 69.2, 61.8, 59.0, 14.1.

MALDI-MS, M^+ 1708.3 (calcd 1708.5).

MTT assay

K562 human erythroleukemia cells (ATCC) were cultured in 25 cm^2 culture flasks containing RPMI 1640 supplemented with heat inactivated 10 % fetal bovine serum, 2 mM L-glutamine, 100 units.mL^{-1} penicillin G and 100 μg.mL^{-1}streptomycin at 37^0C in a humidified incubator containing 5% CO_2. Dye was dissolved in RPMI 1640 and test concentrations were prepared daily.

The methlythiazolyltetrazolium (MTT) assay was used to evaluate cell viability.[S1] Briefly, 50 μl cell suspensions containing 4 x 10^4 K562 cells were seeded in 96-well round-bottom plates (Costar, Cambridge, MA) and varying concentrations of dye (25 nM-500nM) were added in 50 μl into each well. All concentrations were studied in quadriplicate.

Then, cells were kept either in dark or under illumination with a red (625 nm) LED array at 2.5 mW/cm^2 fluence rate for a period of 4 h at 37^0C in a humidified incubator containing 5% CO_2. After 4 h of incubation, 25 μl of MTT solution (1mg/ml final concentration) (Sigma Chemical Co., St. Louis, MO) were added to each well, and the plates were incubated for a further 4 h. The formazan precipitate was solubilized by adding 80 μl lysing buffer (pH=4.7) composed of 23% SDS (sodium dodecyl sulfate) dissolved in a solution of 45% N,N- dimethylformamide. After an overnight incubation at 37^0 C, the optical densities (OD) were read at 570 nm using a microplate reader (Spectramax Plus, Molecular Devices, Sunnyvale, California,USA). Cells incubated in culture medium alone served as a control for cell viability (nontreated wells) either in irradiated plate or in plate that was kept in dark. Cell viability (%) was calculated as (OD of treated wells/OD of nontreated cells) x 100.

In order to evaluate the cytotoxicity of dye after 24 h incubation, 4 h irradiated plates were kept in dark for a further 20 h and then MTT solution was added for measuring cell viability. In addition, plates kept in dark for 4 h were also incubated for a further +20 h for control.

Cell Culture

K562 cells (human chronic myelogenous leukemia cell line, ATCC) were maintained in RPMI-1640 culture medium supplemented with 10% heat-inactivated fetal bovine serum, 2 mM L-glutamine, 100 IU/ml penicillin and 100 μg/ml streptomycin at 37^0 C in a humidified incubator containing 5% CO_2 .

Preparation of stock solutions for microscopy

A stock solution of propidium iodide (PI) (Sigma P-4170) was prepared in distilled water, and used at a concentration of 0.5 mg/ml. Acridine orange (AO) was dissolved in phosphate buffered saline at a concentration of 100 μg/mL. One tablet of phosphate buffered saline (PBS) (Amresco) was dissolved in the distilled water to yield a pH=7.4 buffered solution.

References:

S1. Hansen, M.B.; Nielsen, S. E.; Berg, K. **1989,** *J Immunol. Methods***,** *119*, 203-210.

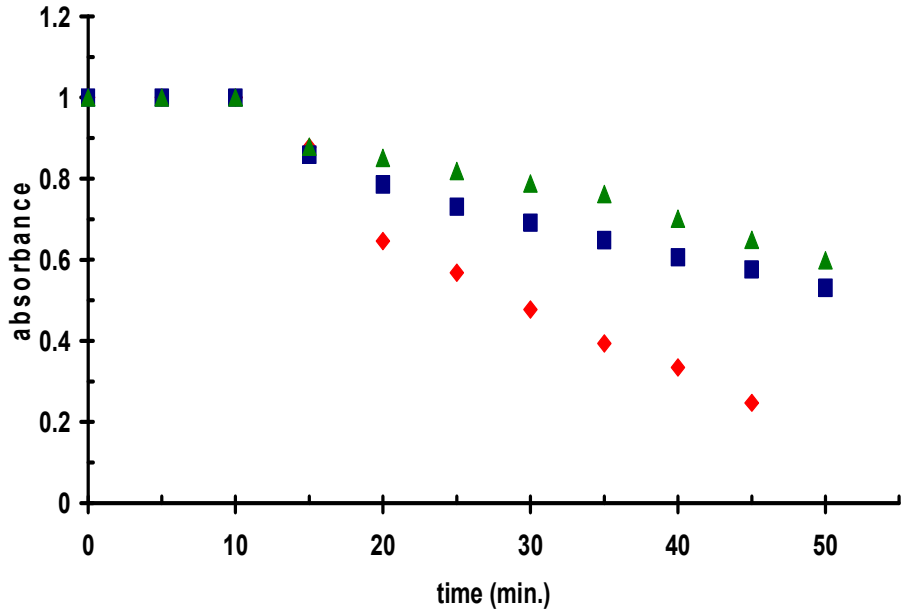
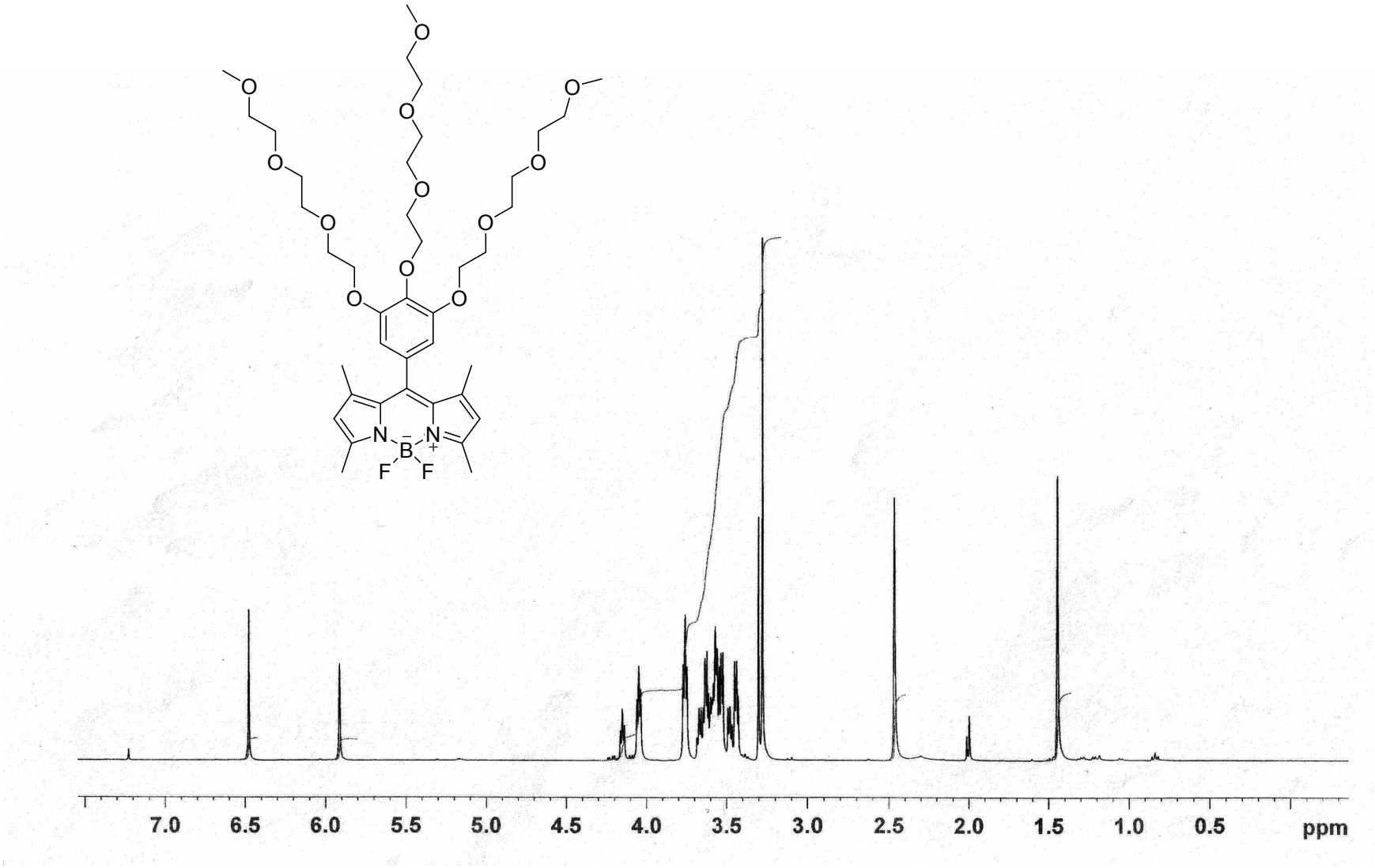


Figure S1. Bleaching of 1,3-diphenylisobenzofuran (50 μM) in the presence of the sensitizers **1-3** (numbering is as it is in the article) at 9 nM concentration in isopropanol solution. For the first 10 minutes the solutions were kept in dark, as irradiation at 625 nm starts at the 10th minute, rapid degradation of the singlet oxygen trap starts. Triangles, compound **3**; squares, compound **2**; and diamonds, compound **1**.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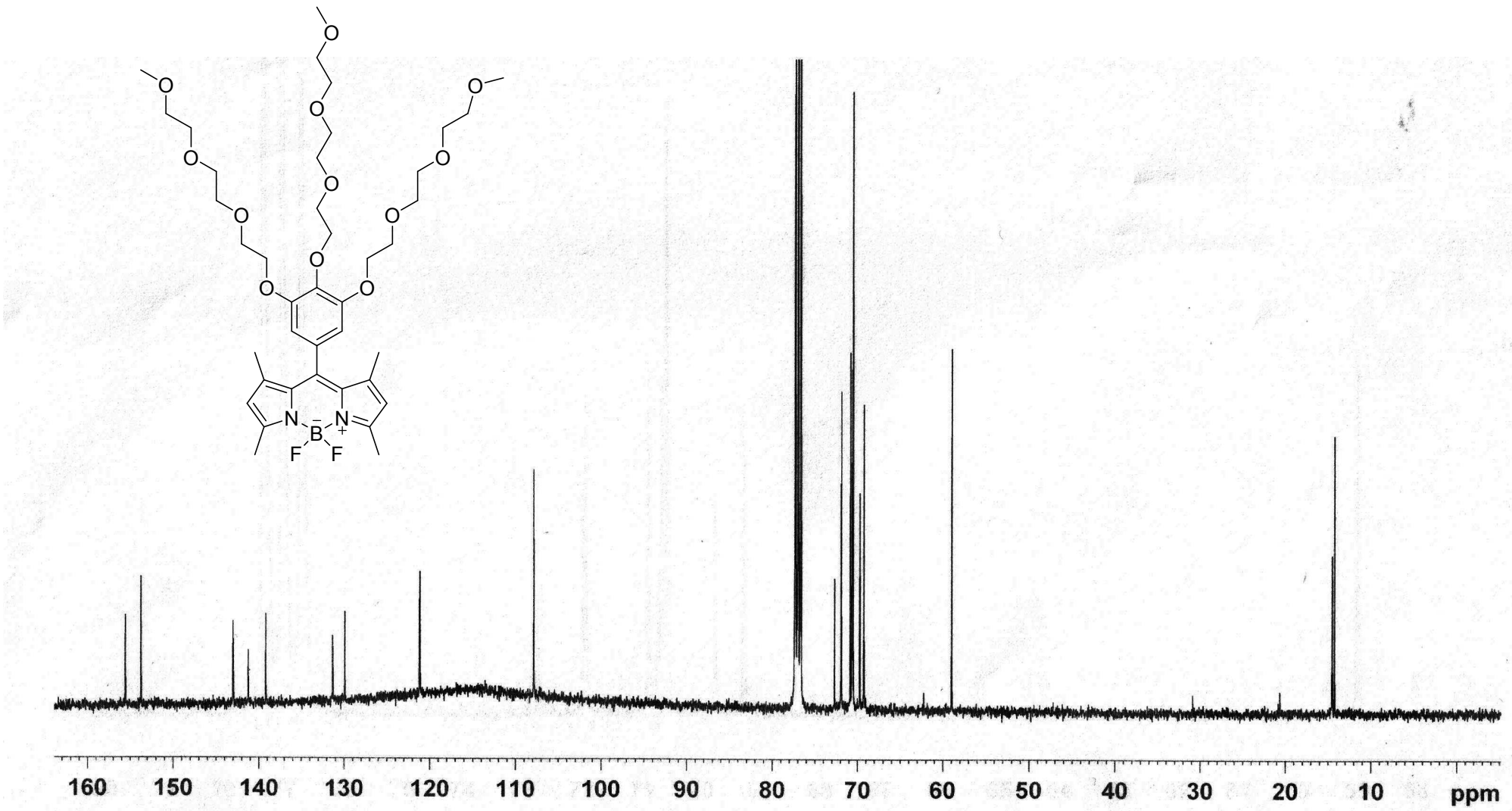
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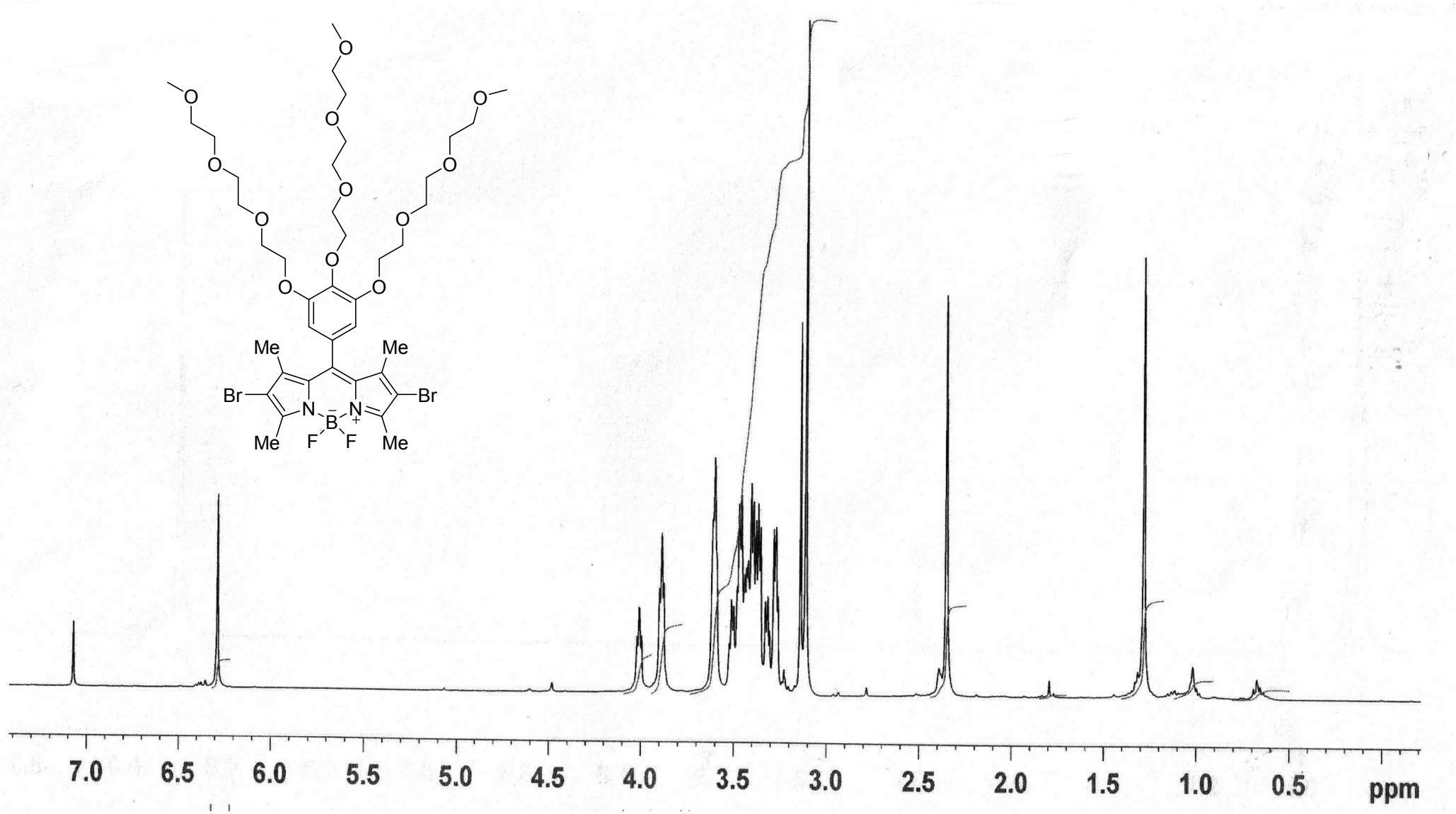
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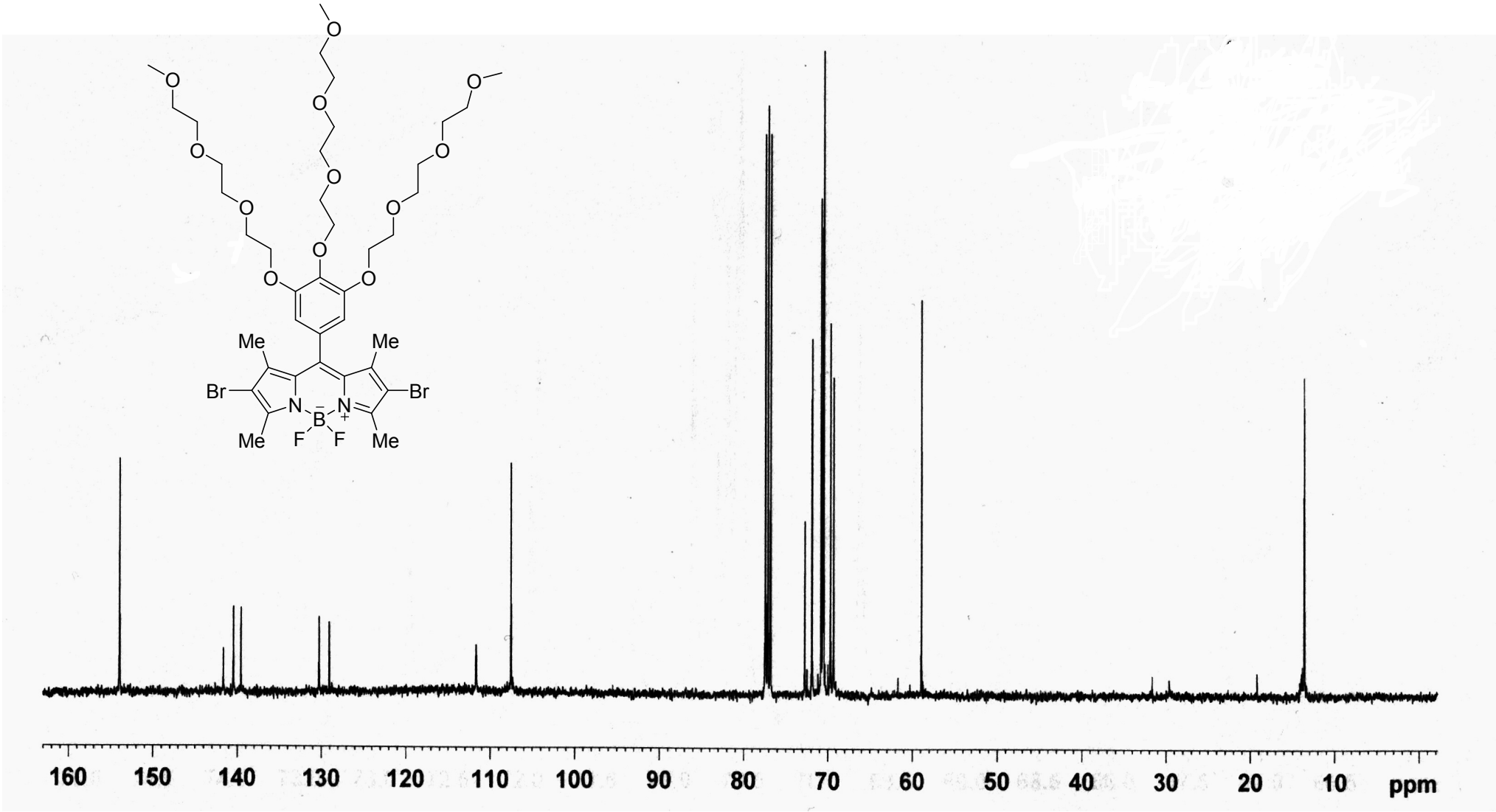
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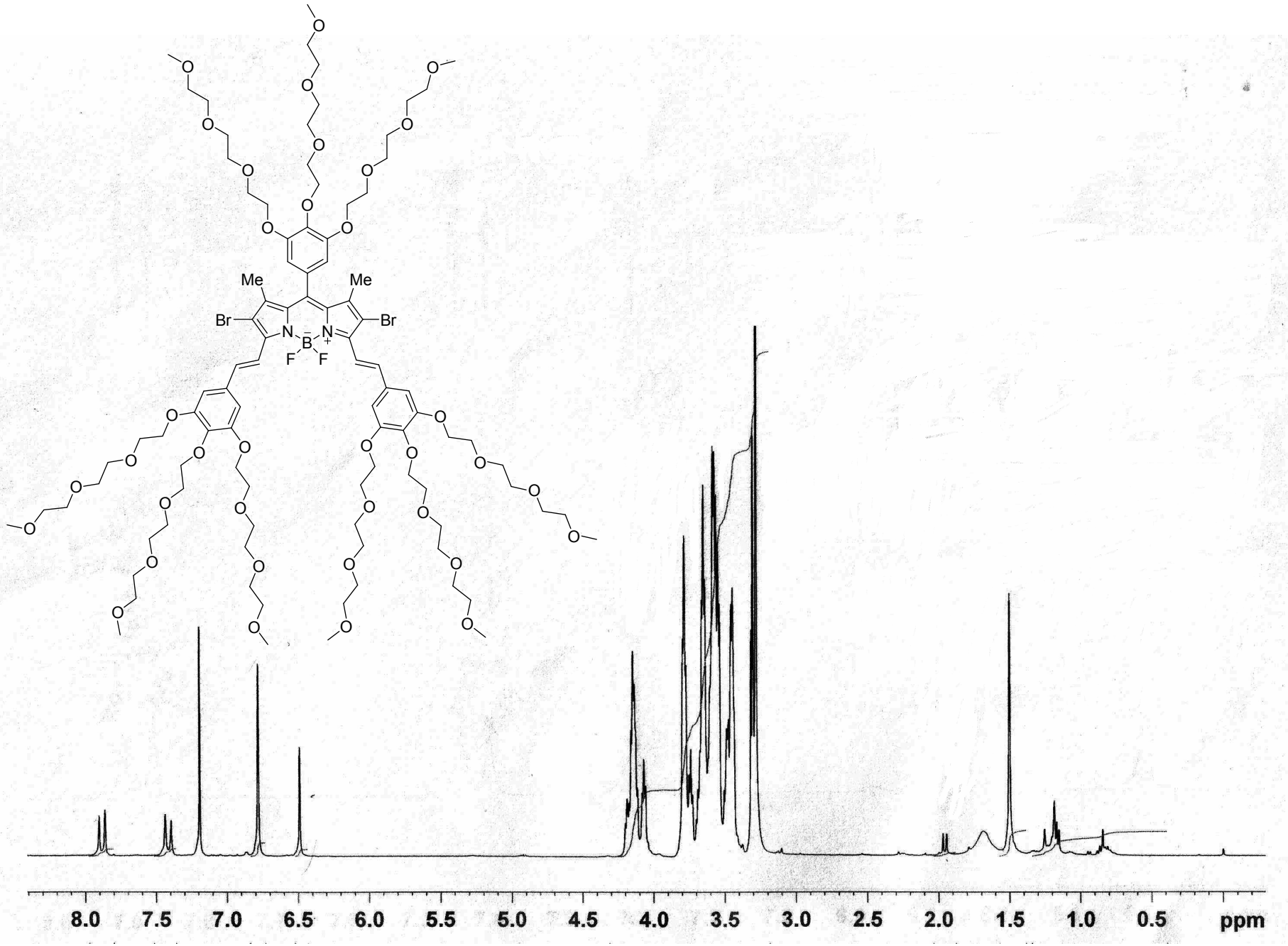
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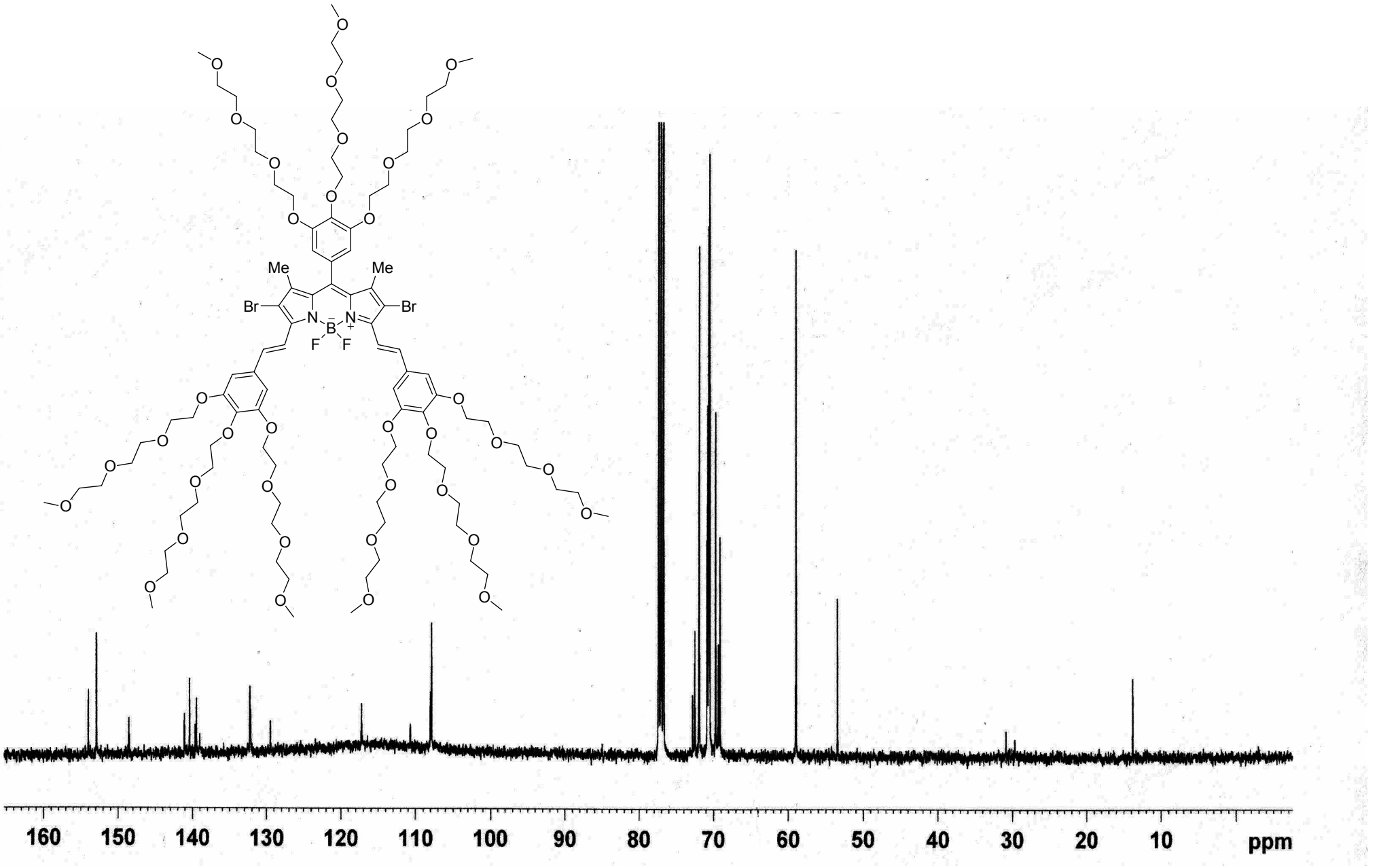
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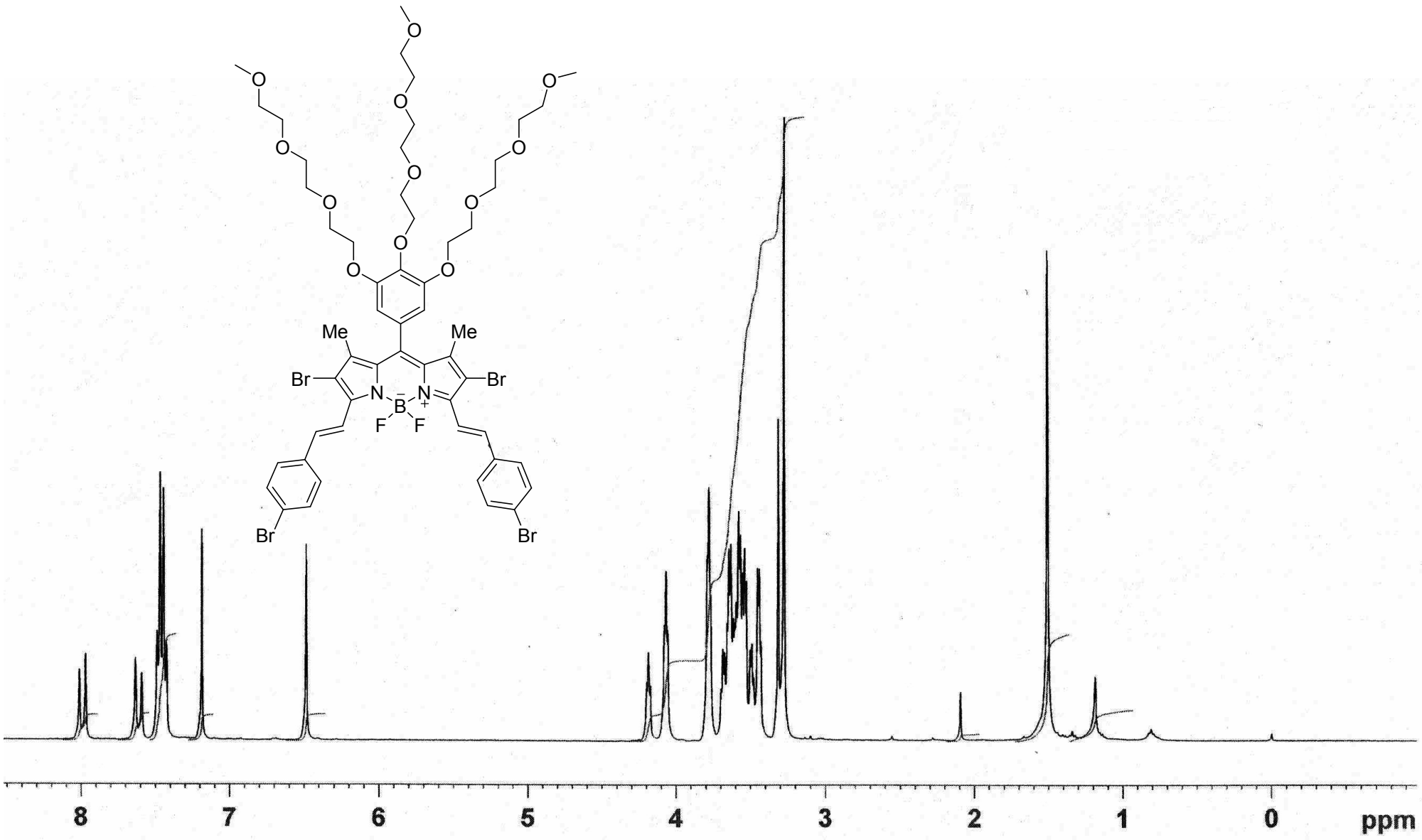
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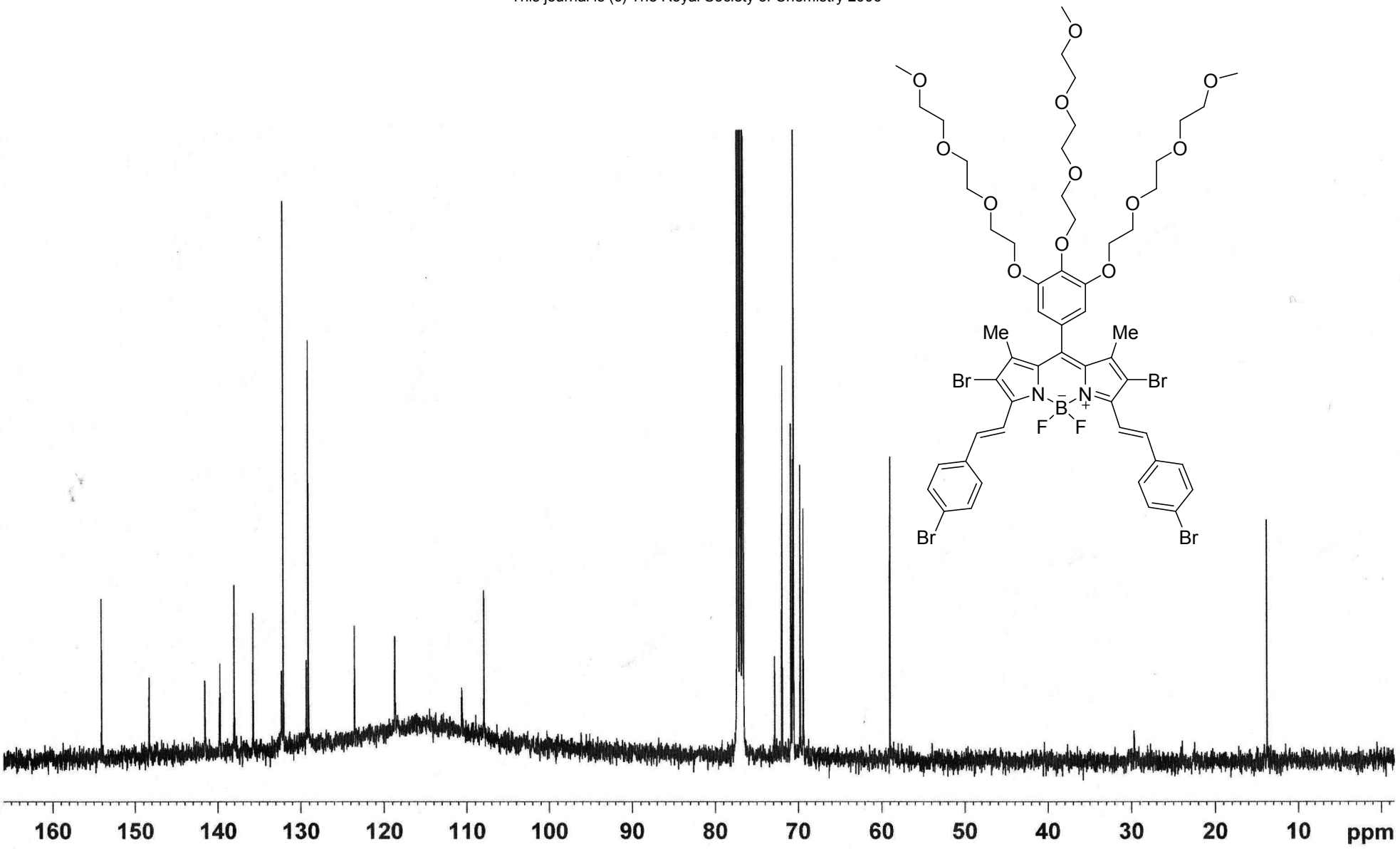
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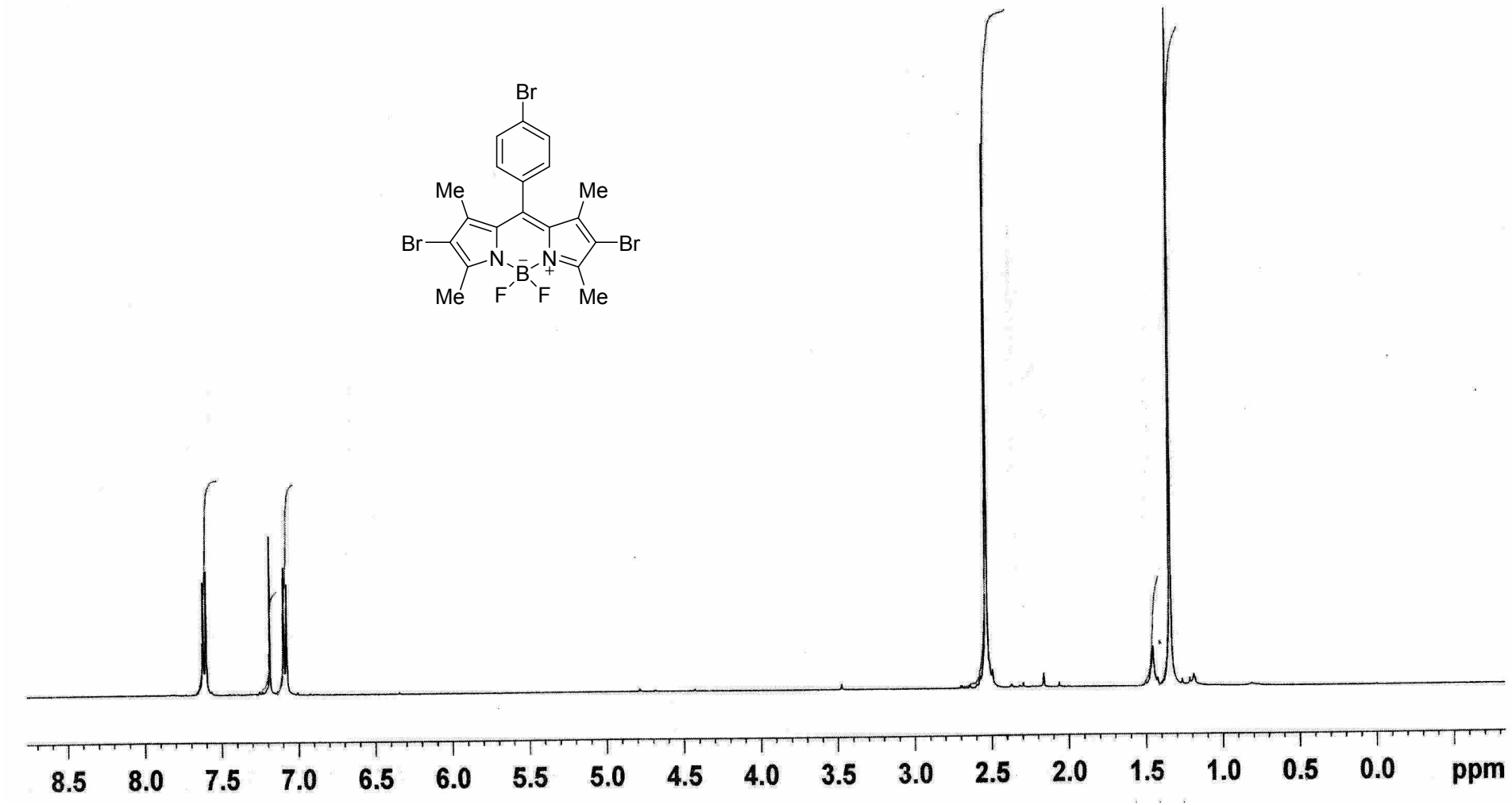

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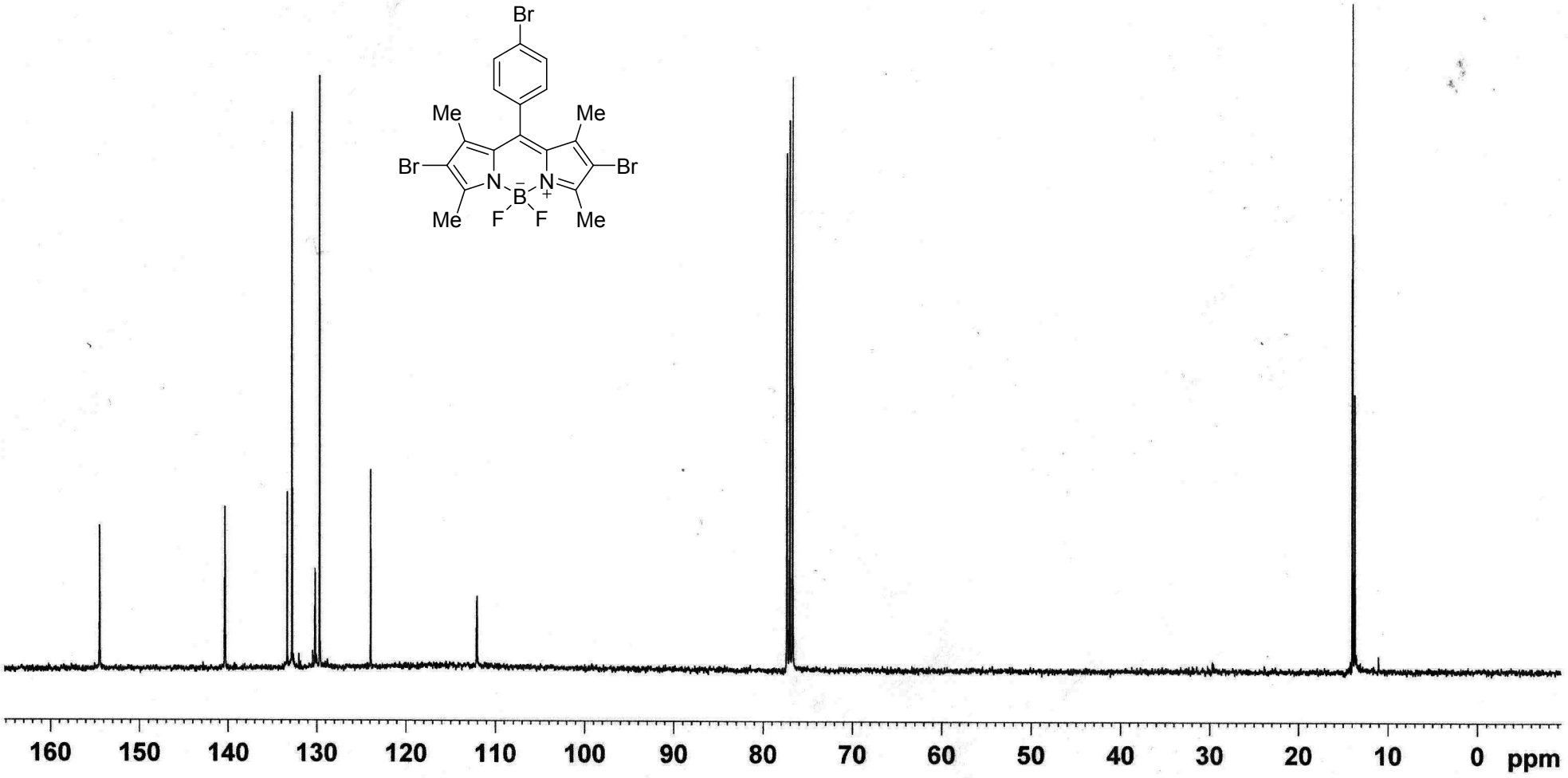

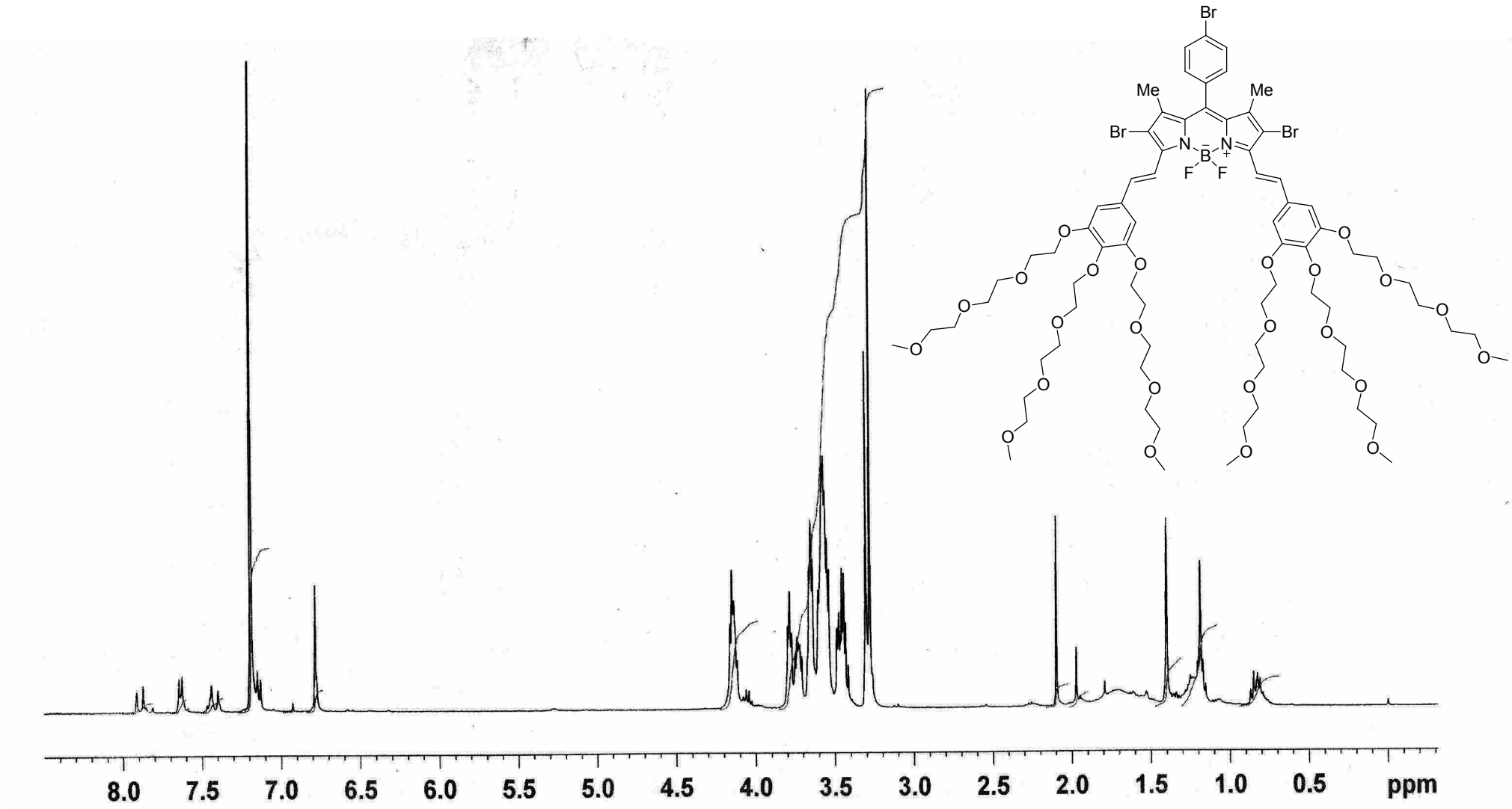
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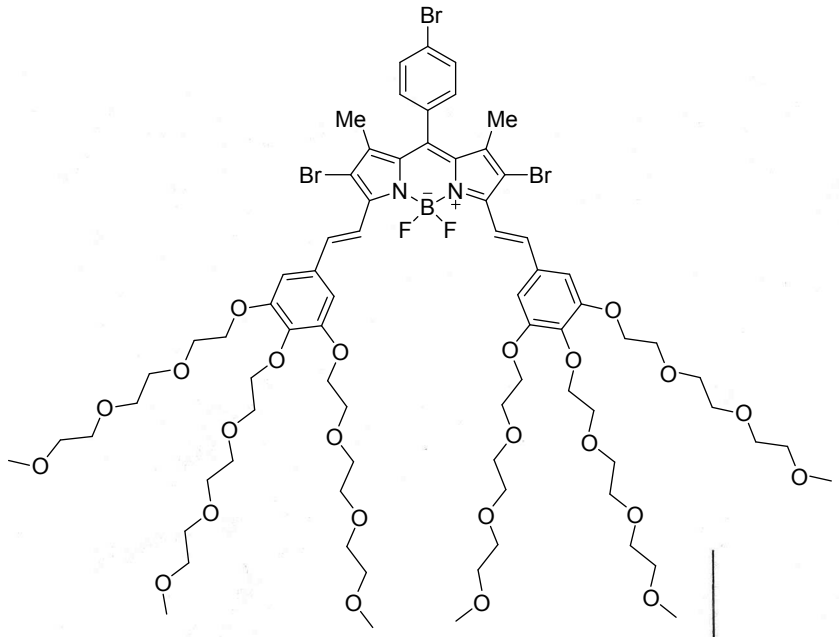
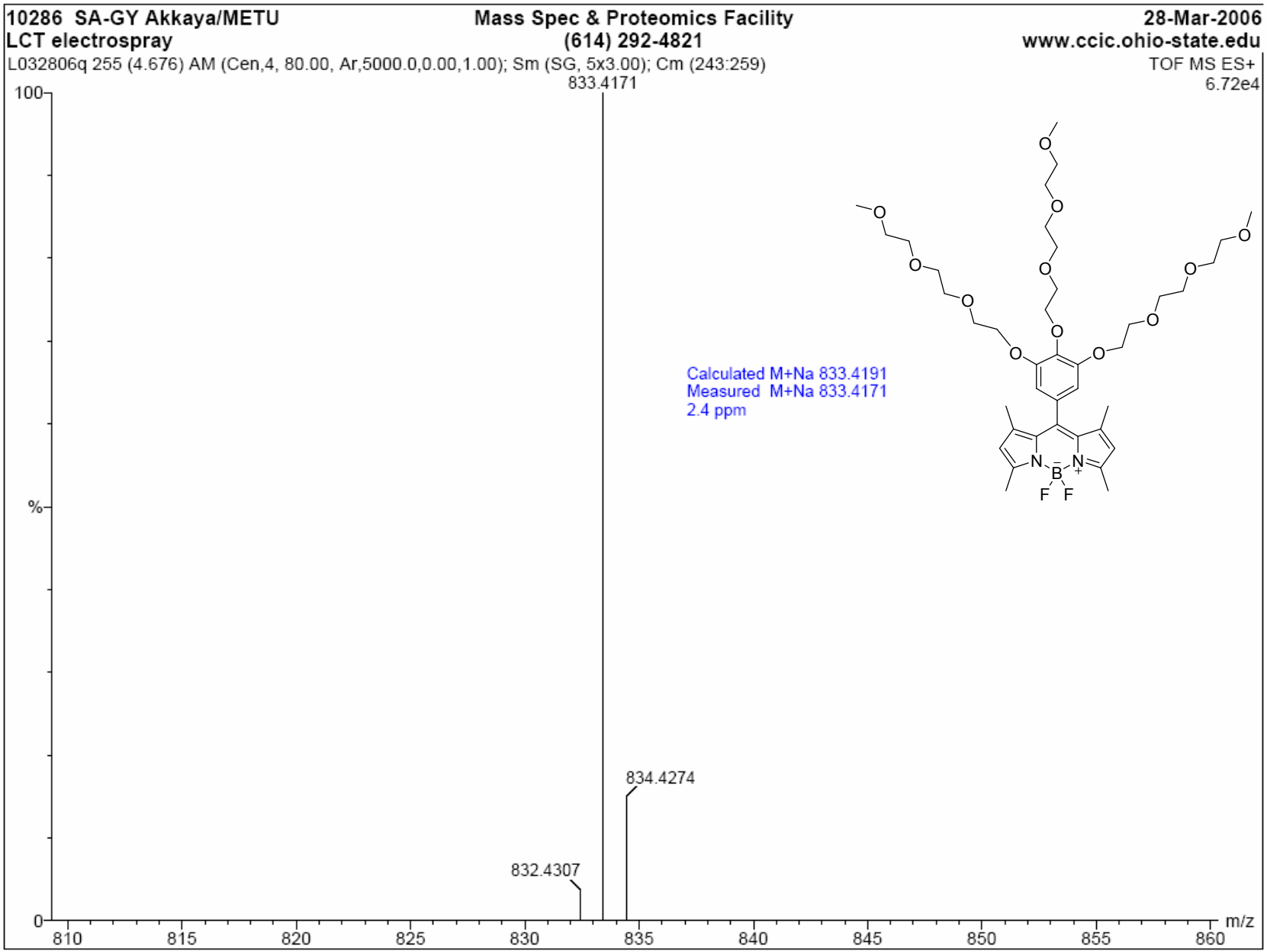
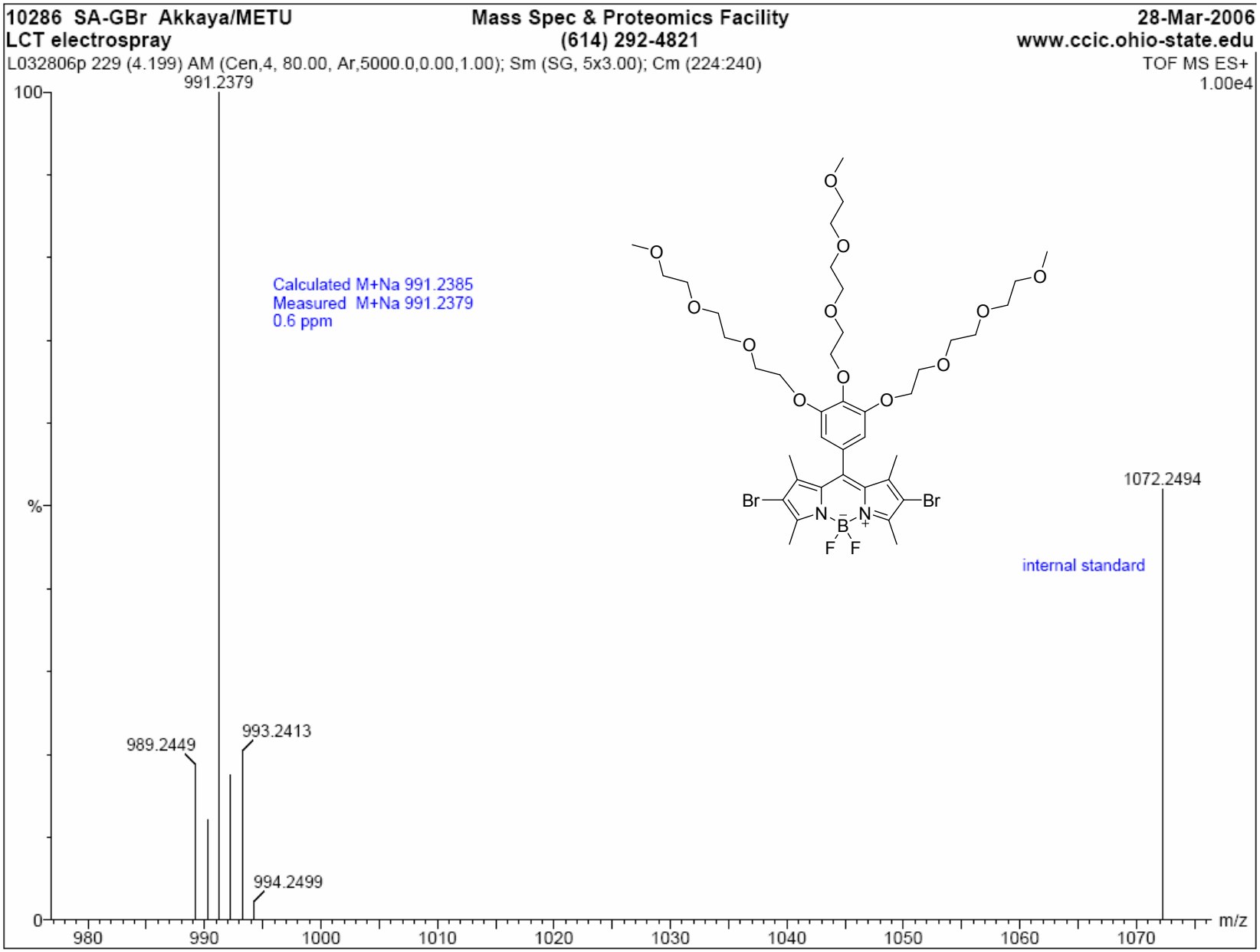
F
F
O
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm

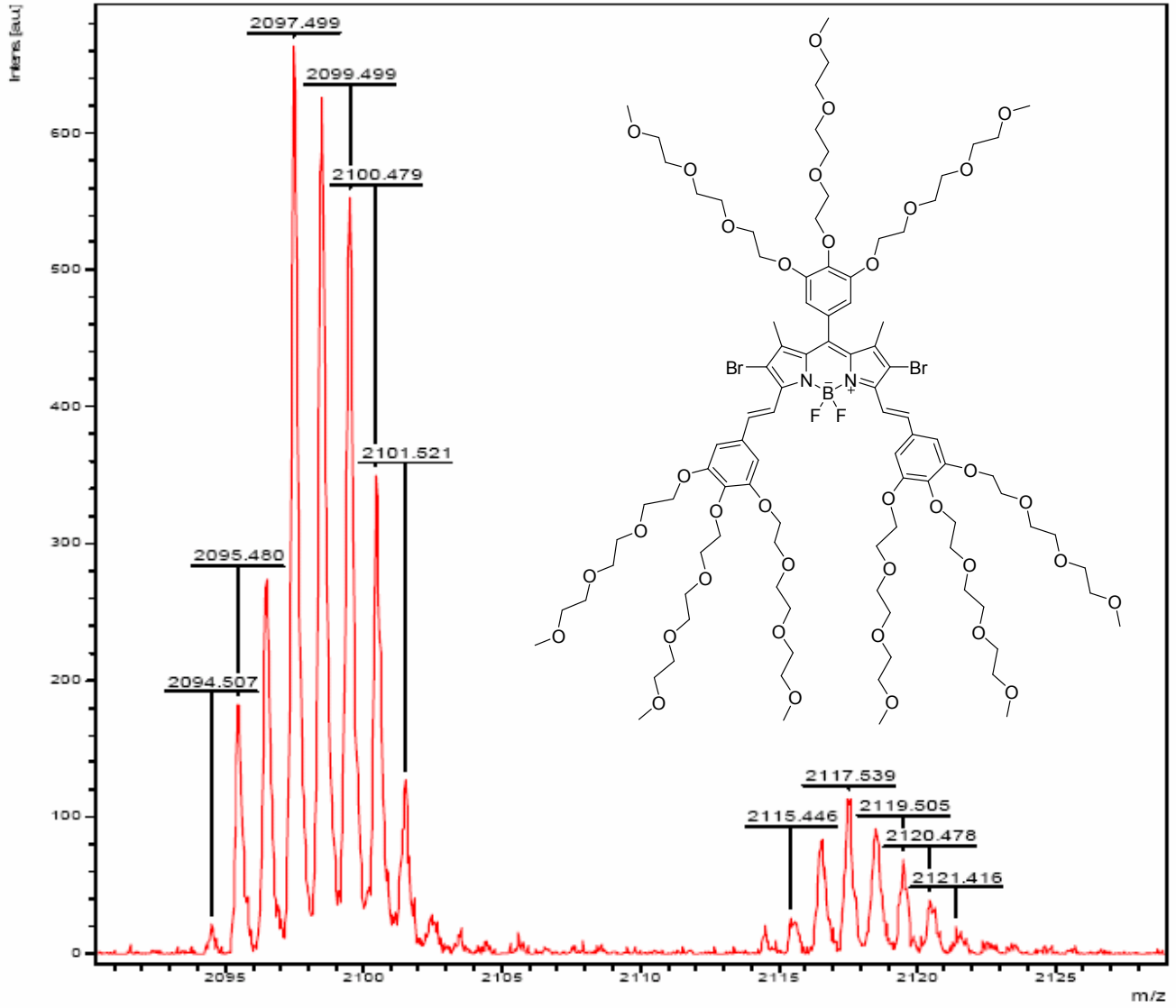

Br
Me
Me
Br
Br
N
B
N
F
F

160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm


10286 SA-GY Akkaya/METU
LCT electrospray
Mass Spec & Proteomics Facility
(614) 292-4821
28-Mar-2006
www.ccic.ohio-state.edu
L032806q 255 (4.676) AM (Cen,4, 80.00, Ar,5000.0,0.00,1.00); Sm (SG, 5x3.00); Cm (243:259)
TOF MS ES+
6.72e4
833.4171
100
%
0
834.4274
832.4307
Calculated M+Na 833.4191
Measured M+Na 833.4171
2.4 ppm
810
815
820
825
830
835
840
845
850
855
860
m/z


10286 SA-GBr Akkaya/METU
LCT electrospray
Mass Spec & Proteomics Facility
(614) 292-4821
28-Mar-2006
www.ccic.ohio-state.edu
L032806p 229 (4.199) AM (Cen,4, 80.00, Ar,5000.0,0.00,1.00); Sm (SG, 5x3.00); Cm (224:240)
TOF MS ES+
1.00e4
991.2379
Calculated M+Na 991.2385
Measured M+Na 991.2379
0.6 ppm
1072.2494
internal standard
989.2449
993.2413
994.2499
100
%
0
980
990
1000
1010
1020
1030
1040
1050
1060
1070
m/z
Br
Br
N
B
N
F
F
O

D:\DATA\MALDI\M032306\10285 SA-03 Akkaya



m/z	SN	Quality Fac.	Res.	Intens.	Area
2019.566	87.2	4373	7516	133.00	41
2038.603	11.8	881	25103	18.00	3
2069.491	49.8	3222	8872	76.00	24
2094.507	14.4	832	13405	22.00	5
2095.480	119.3	6682	7731	182.00	58
2096.489	179.6	10300	7917	274.00	91
2097.499	434.7	23002	7441	663.00	224
2098.501	410.4	21637	7058	626.00	222
2099.499	362.5	18500	6612	553.00	203
2100.479	229.5	12711	7159	350.00	124
2101.521	83.3	4004	6261	127.00	48
2115.446	17.0	561	5508	26.00	9
2116.541	55.1	2914	5919	84.00	34
2117.539	74.1	3823	7468	113.00	40
2118.528	60.3	3593	5969	92.00	40
2119.505	45.2	1611	5521	69.00	24
2120.478	25.6	1409	6445	39.00	16
2121.416	13.1	3770	35272	20.00	7
2139.516	177.0	9545	7195	270.00	98
2273.547	49.2	2729	6753	75.00	31

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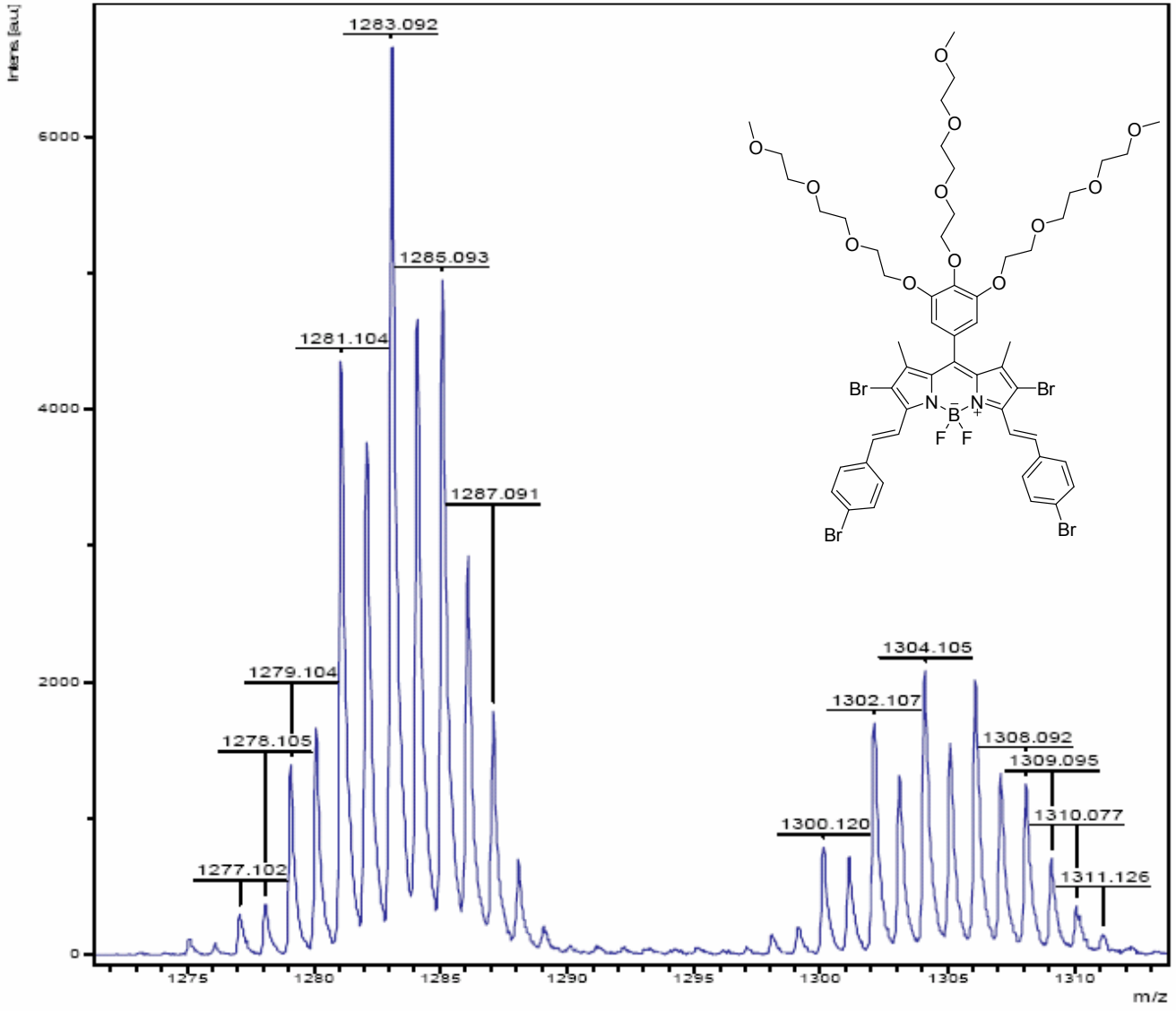

m/z	SN	Quality Fac.	Res.	Intens.	Area
1175.201	27.3	2511	6293	77.00	16
1205.199	589.4	90143	6725	1660.00	404
1224.182	119.7	17021	7570	337.00	71
1255.108	310.3	43837	7083	874.00	204
1277.102	105.5	15069	5485	297.00	88
1278.105	132.4	17016	5510	373.00	110
1279.104	495.0	69742	6052	1394.00	388
1280.106	591.5	74703	5591	1666.00	489
1281.104	1545.6	227008	5489	4353.00	1359
1282.108	1333.6	185394	5070	3756.00	1267
1283.092	2361.8	345034	5239	6652.00	2224
1284.095	1653.5	235828	5145	4657.00	1591
1285.093	1756.5	249497	5144	4947.00	1685
1286.092	1039.3	149374	5281	2927.00	983
1287.091	635.6	97820	5599	1790.00	592
1299.123	73.1	10679	4919	206.00	71
1300.120	281.2	41558	5994	792.00	244
1301.111	254.9	33610	5221	718.00	233
1302.107	604.7	86500	5259	1703.00	549
1303.114	466.5	61071	4988	1314.00	443
1304.105	738.9	104247	5057	2081.00	707
1305.106	551.8	70613	4999	1554.00	527
1306.099	715.4	99636	5014	2015.00	683
1307.101	472.2	66492	5082	1330.00	462
1308.092	446.3	57442	4803	1257.00	423
1309.095	253.5	35253	5193	714.00	240
1310.077	127.5	16540	4139	359.00	142
1311.126	52.9	3787	4351	149.00	50
1325.123	1678.0	212340	3442	4726.00	2151
1341.134	339.8	42663	3749	957.00	402
1381.246	73.1	9502	5535	206.00	64
1415.153	68.5	8300	6903	193.00	52
1435.104	80.6	9209	4940	227.00	74
1459.153	487.8	58641	4689	1374.00	507
1475.163	88.4	11341	5800	249.00	85
1569.133	12.1	1928	7063	34.00	12

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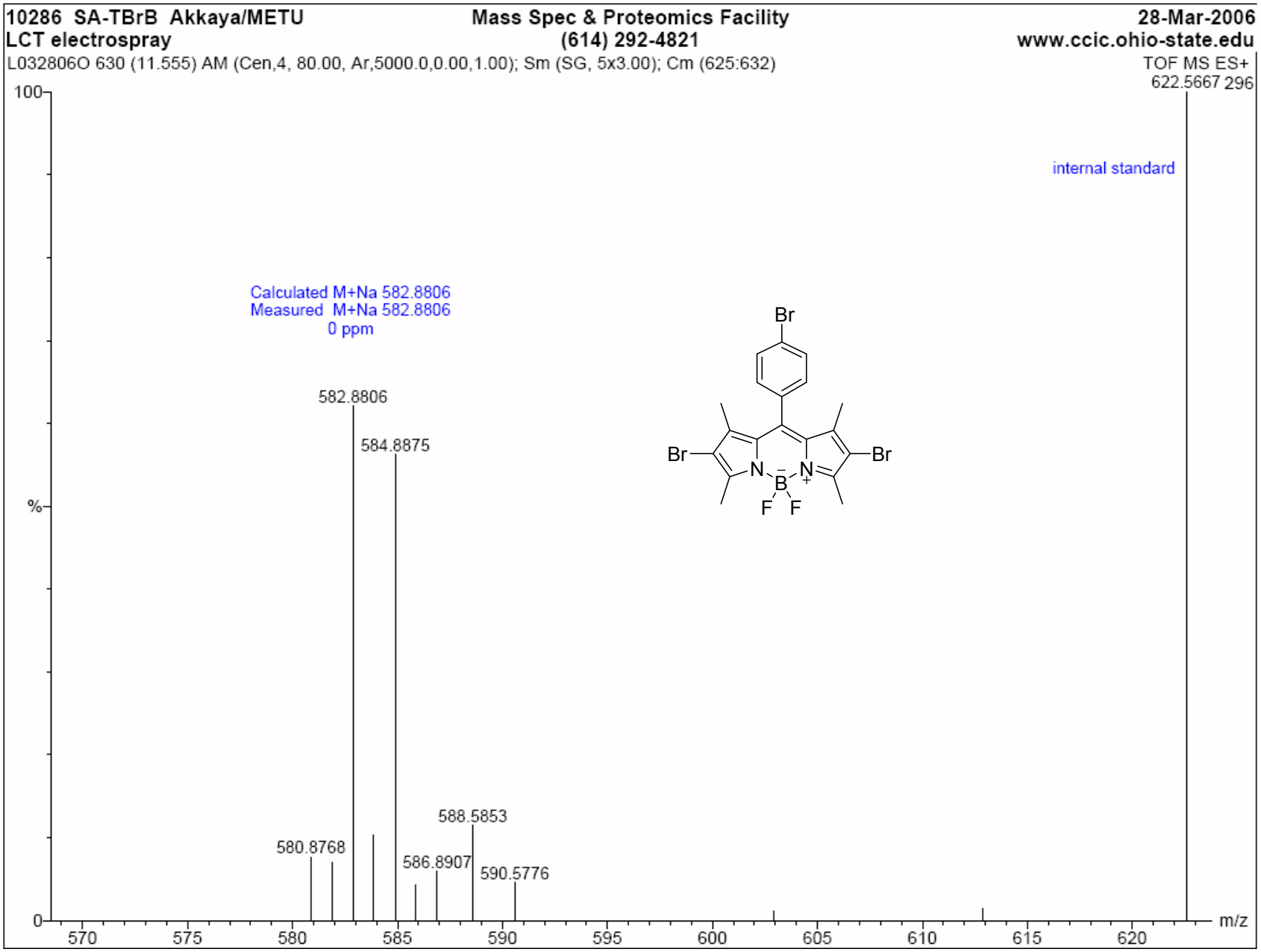
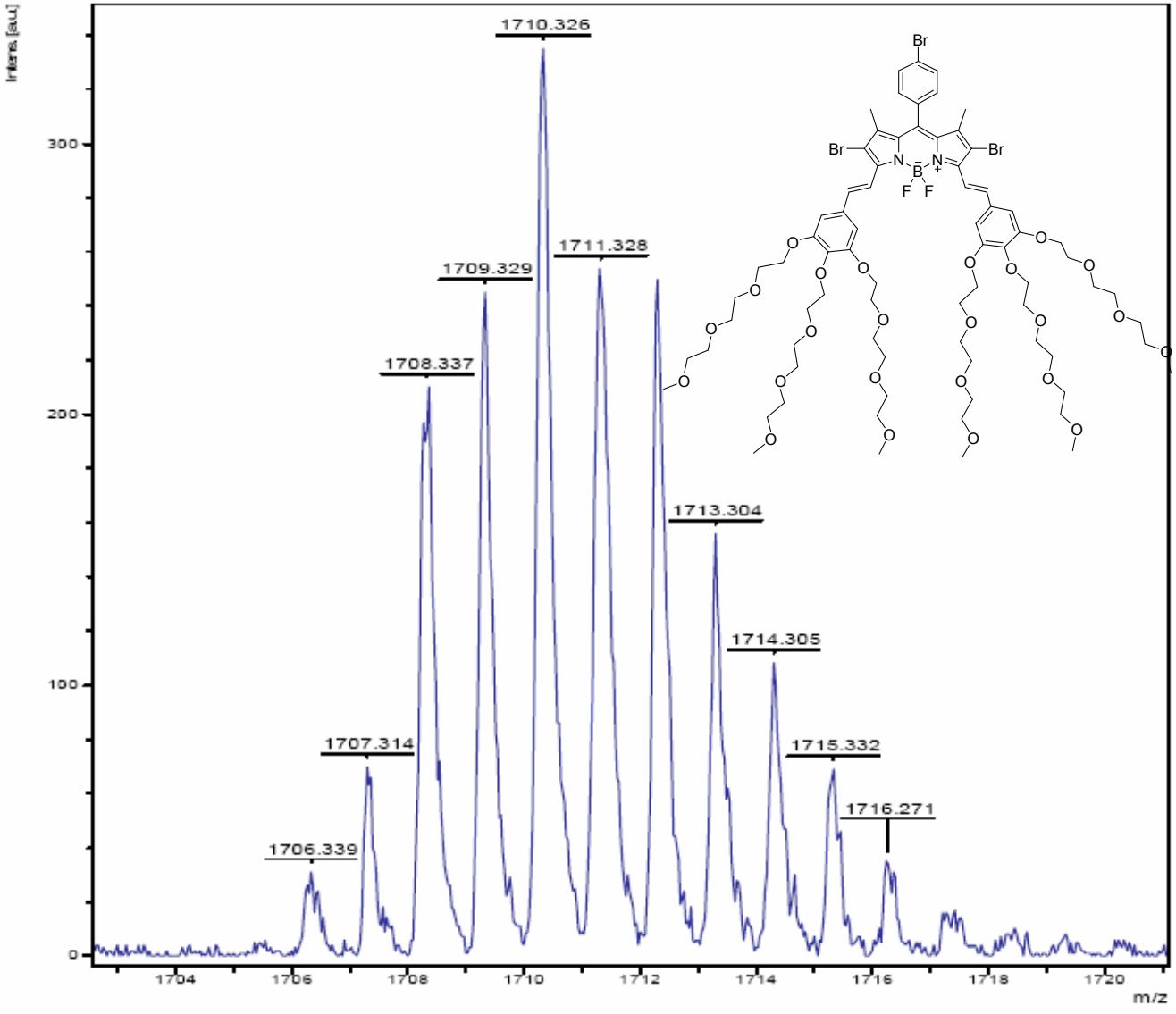

10286 SA-TBrB Akkaya/METU
LCT electrospray
Mass Spec & Proteomics Facility
(614) 292-4821
28-Mar-2006
www.ccic.ohio-state.edu
L032806O 630 (11.555) AM (Cen,4, 80.00, Ar,5000.0,0.00,1.00); Sm (SG, 5x3.00); Cm (625:632)
TOF MS ES+
622.5667 296
internal standard
Calculated M+Na 582.8806
Measured M+Na 582.8806
0 ppm
582.8806
584.8875
580.8768
588.5853
586.8907
590.5776
100
%
0
570
575
580
585
590
595
600
605
610
615
620
m/z
Br
Br
Br
N
B
N
F
F

D:\DATA\MALDI\M032306\10285 SA-02 Akkaya



m/z	SN	Quality Fac.	Res.	Intens.	Area
1611.393	145.0	11785	11500	285.00	50
1630.377	18.8	1136	8918	37.00	8
1653.392	42.2	3380	8444	83.00	19
1663.304	119.5	10462	9781	235.00	51
1691.296	753.3	65204	8432	1481.00	396
1706.339	15.8	891	5963	31.00	9
1707.314	35.6	2402	8112	70.00	17
1708.337	106.8	8728	6731	210.00	65
1709.329	124.6	8993	6906	245.00	72
1710.326	170.4	13347	6177	335.00	112
1711.328	129.2	9676	6011	254.00	86
1712.314	127.2	9312	6639	250.00	75
1713.304	79.3	7139	8085	156.00	46
1714.305	54.9	4507	7399	108.00	33
1715.332	35.1	2342	7147	69.00	19
1716.271	17.8	1617	7225	35.00	11
1733.313	565.6	45320	5886	1112.00	417
1749.316	71.2	4963	5764	140.00	50
1867.371	156.1	10946	6458	307.00	106

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