

SUPPORTING INFORMATION

Signal Ratio Amplification *via* Modulation of Resonance Energy Transfer: Proof of Principle in an Emission Ratiometric Hg(II) Sensor

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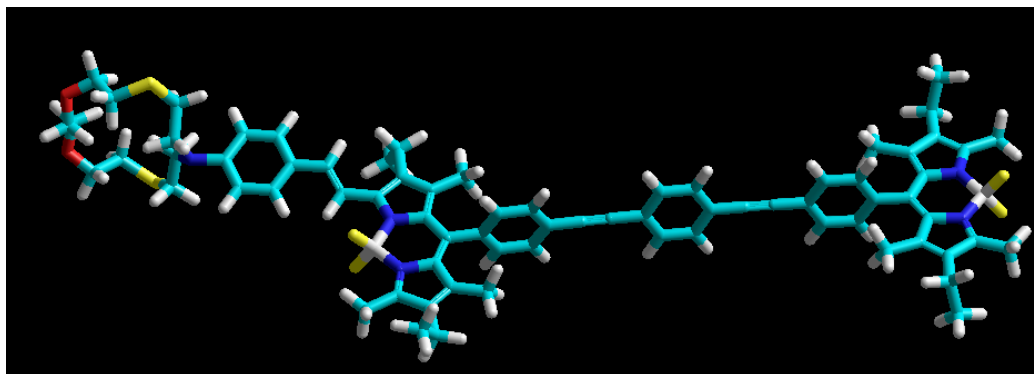


Figure 1. Energy minimized (HyperChem v. 7.5, PM3 semiempirical module) structure of compound **3c**. Calculated B-B distance 25.5 Å.

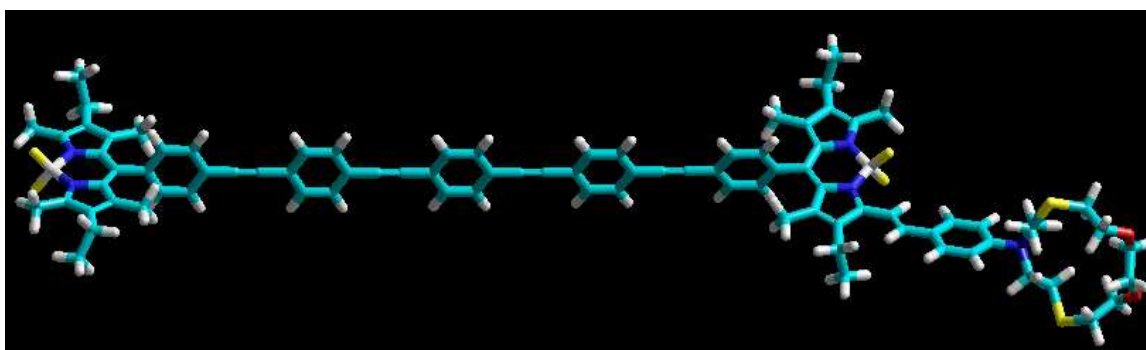


Figure 2. Energy minimized (HyperChem v. 7.5, PM3 semiempirical module) structure of compound **4c**. Calculated B-B distance 39.1 Å.

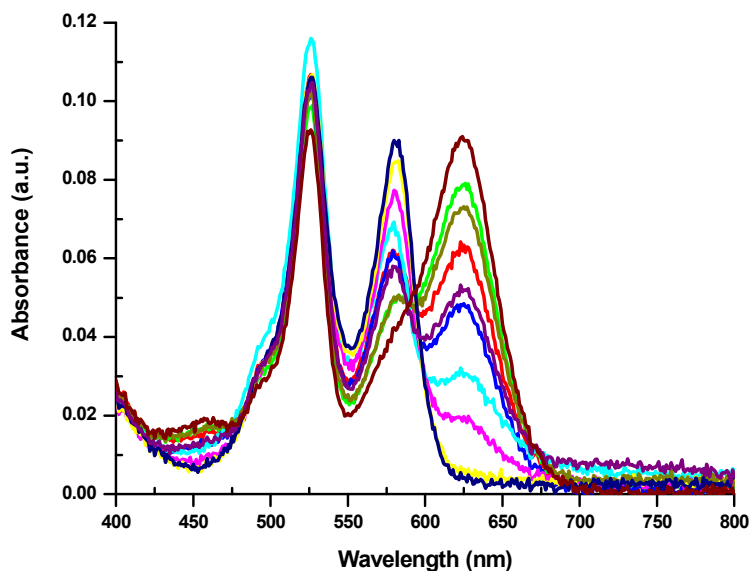


Figure 3. Absorbance spectra of compound **3c** in the presence of increasing Hg(II) concentrations (0, 1.0, 2.0, 3.0, 4.0, 5.0, 10, 15, 20, 25 μM). The concentration of the chemosensor was 1.0 μM .

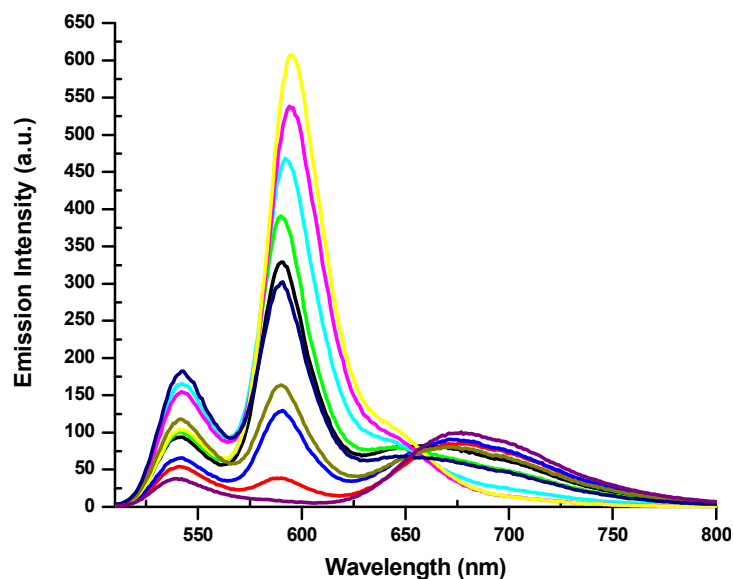


Figure 4. Emission spectra of compound **3c** in the presence of increasing Hg(II) concentrations (0, 1.0, 2.0, 3.0, 4.0, 5.0, 10, 15, 20, 25 μM). Excitation wavelength 500 nm with 5 nm slit widths, and spectra were corrected. The concentration of the chemosensor was 1.0 μM .

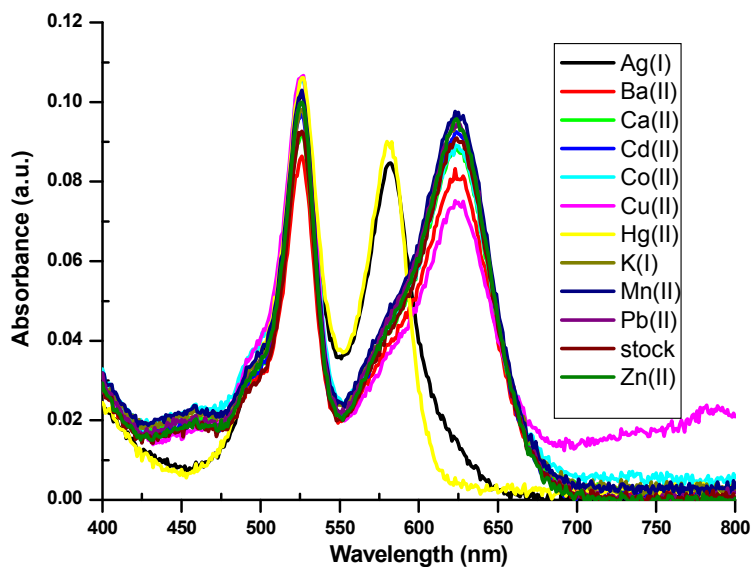


Figure 5. Absorbance spectra of compound **3c** in the presence of various cations (cation concentrations 50 μ M). The concentration of the chemosensor was 1.0 μ M.

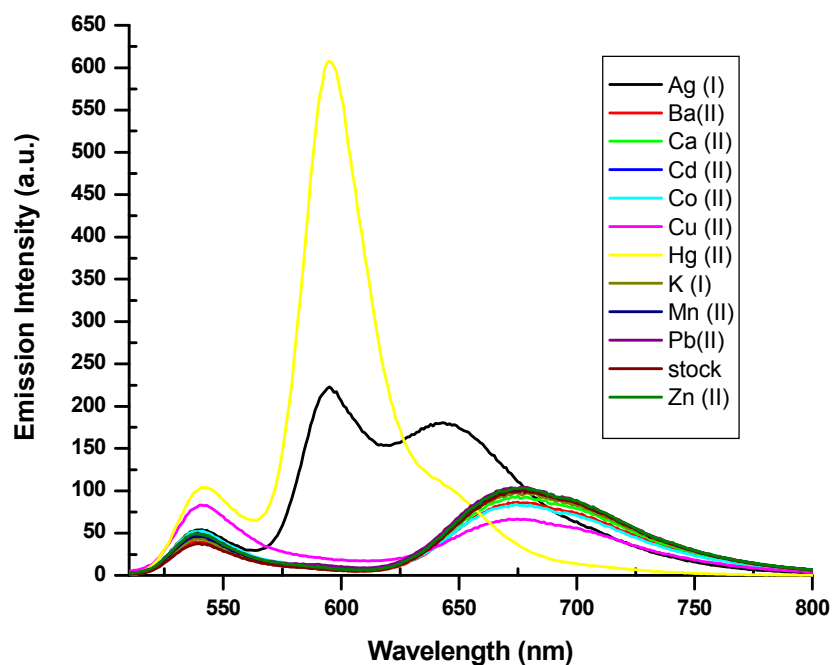


Figure 6. Emission spectra of compound **3c** in the presence of various cations (cation concentrations 50 μ M). Excitation wavelength 500 nm with 5 nm slit widths, and spectra were corrected. The concentration of the chemosensor was 1.0 μ M.

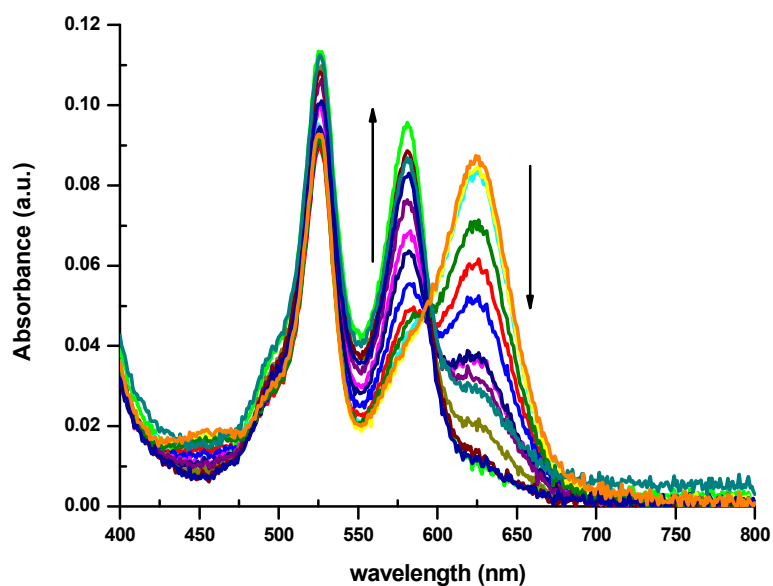


Figure 7. Absorbance spectra of compound **4c** in the presence of increasing Hg(II) concentrations (0, 0.5, 0.75, 1.0, 2.0, 3.0, 4.0, 5.0, 7.5, 10, 12.5, 15, 20, 25 μM). The concentration of the chemosensor was 1.0 μM .

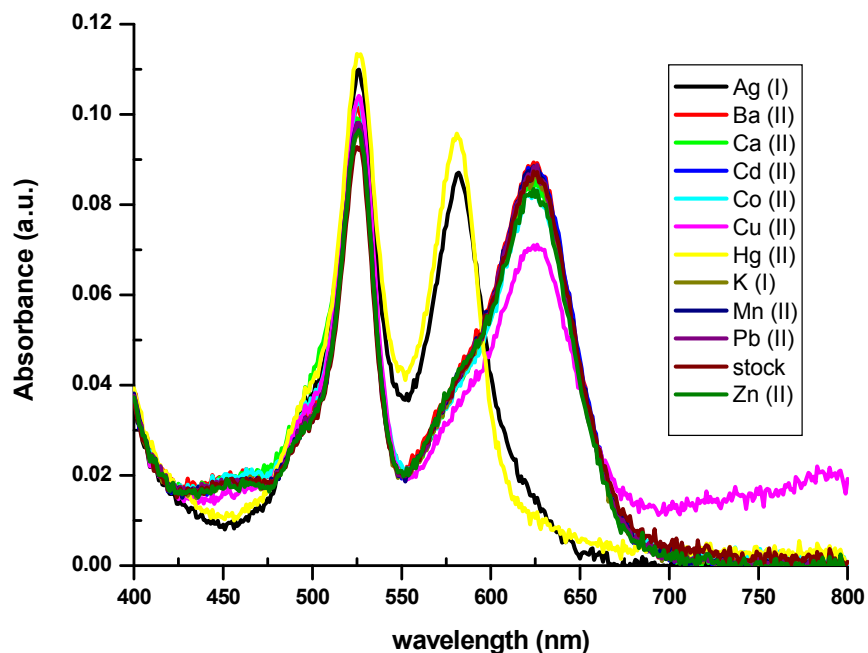


Figure 8. Absorbance spectra of compound **4c** in the presence of various cations (cation concentrations 50 μM). The concentration of the chemosensor was 1.0 μM .

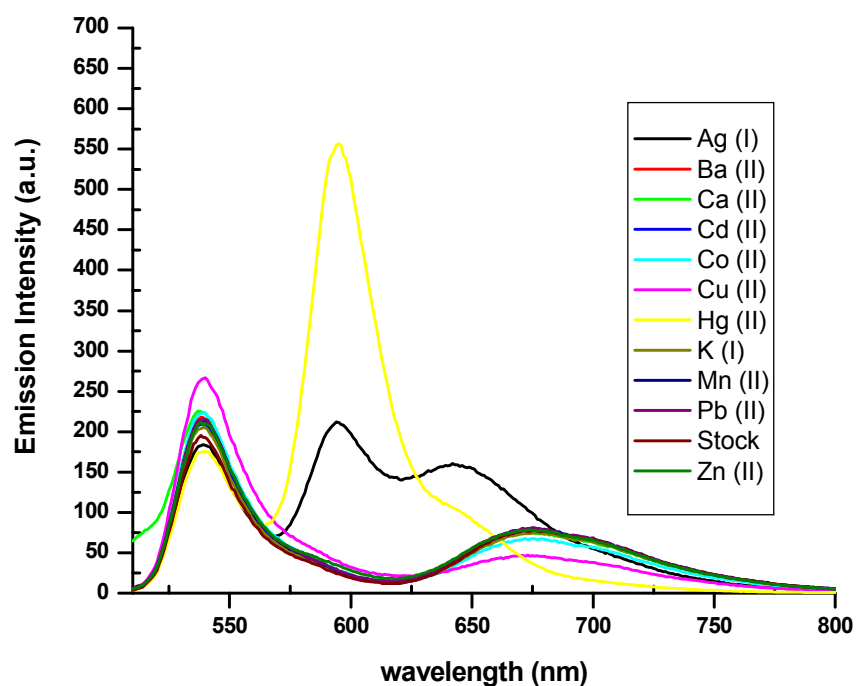


Figure 9. Emission spectra of compound **4c** in the presence of various cations (cation concentrations 50 μM). Excitation wavelength 500 nm with 5 nm slit widths, and spectra were corrected. The concentration of the chemosensor was 1.0 μM .

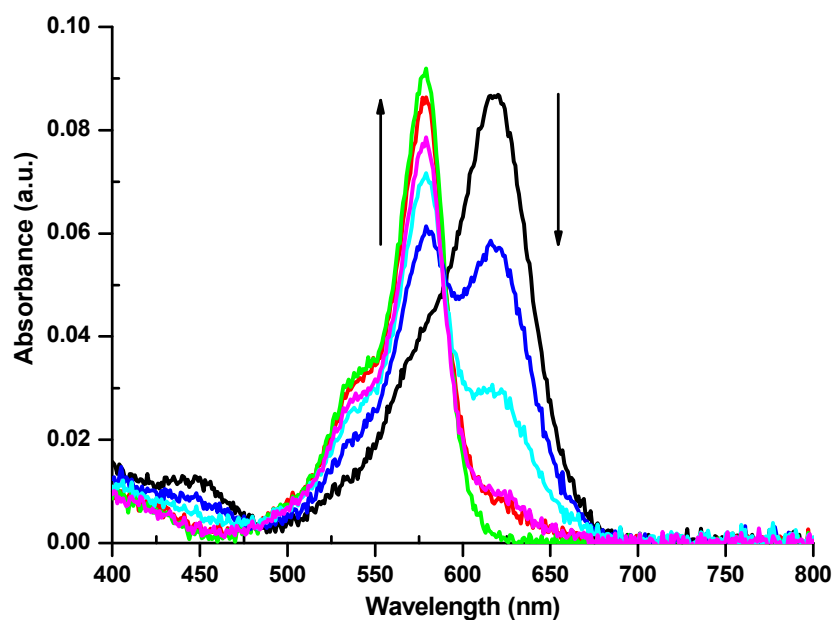


Figure 10. Absorbance spectra of compound **1** (reference compound) in the presence of increasing Hg(II) concentrations (0, 5.0, 10, 15, 20, 25 μM). The concentration of the chemosensor was 1.0 μM .

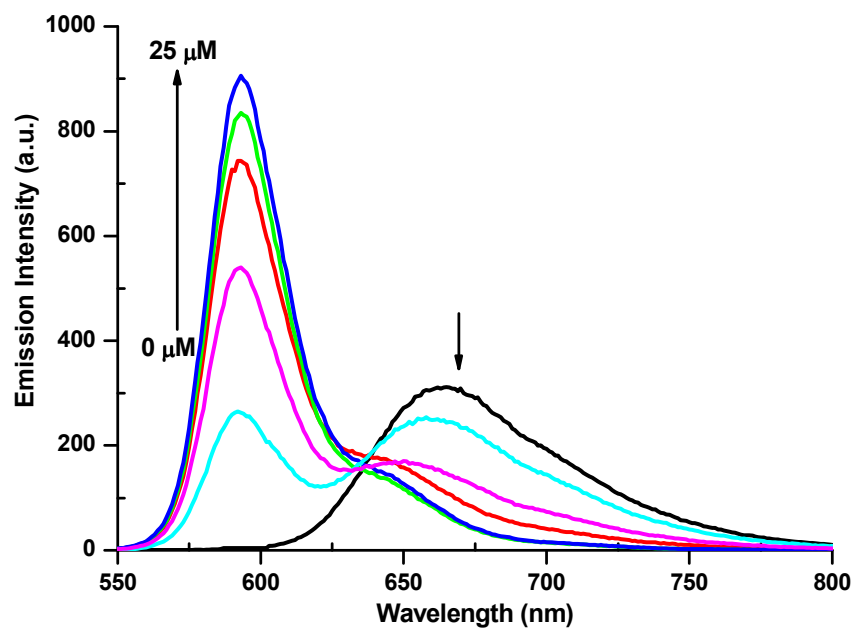


Figure 11. Emission spectra of compound **1** (reference compound) in the presence of increasing Hg(II) concentrations (0, 5.0, 10, 15, 20, 25 μM). Excitation wavelength 500 nm with 5 nm slit widths, and spectra were corrected. The concentration of the chemosensor was 1.0 μM .

Table 1. Quantum yields of the dyes in dilute THF solutions.

Compounds	Quantum yield_dye1	Quantum yield_dye2
3a	0.63	-
3b	0.021	0.17
3c	0.029	0.17
3c+Hg(II)	0.052	0.41
4a	0.59	-
4b	0.097	0.12
4c	0.13	0.13
4c+Hg(II)	0.078	0.35
1	-	0.16
1+Hg(II)	-	0.40

All dyes were excited at 500 nm, and all spectra were corrected, excitation and emission slits were both set at 5 nm. Rhodamine 6G was used as the reference compound in quantum yield measurements, Quantum yield of rhodamine is 0.95 in ethanol. (see: Du, H.; Fuh, R. A.; Li, J.; Corkan, A.; Lindsey, J. S. Photochem. Photobiol. 1998, 68, 141-142.) Corrections for refractive indices were done. The last column refers to the quantum yields of long wavelength emission from the energy acceptor dye.

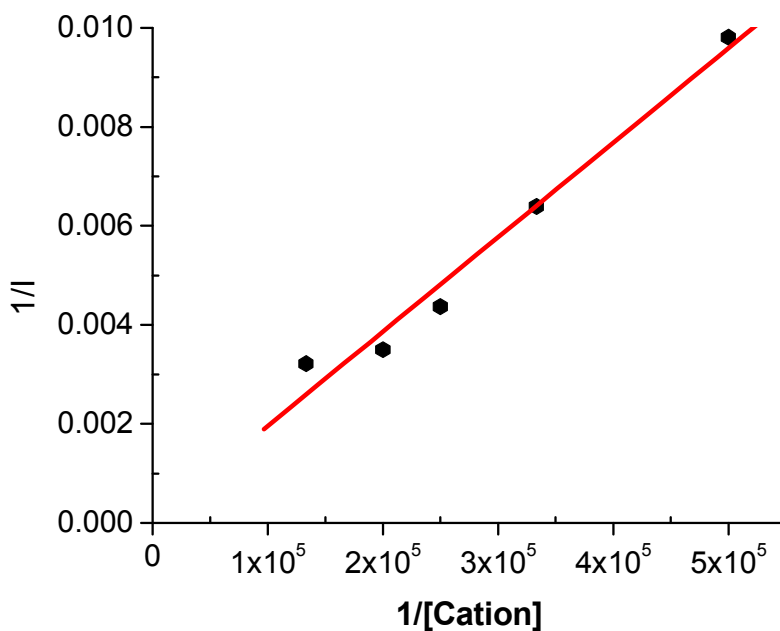


Figure 12. Benesi-Hildebrand analysis of compound **4c** at different Hg(II) concentrations with the emission data collected at 595 nm. Result of the least square analysis follows:

Equation: $Y = A + BX$ $A_0: 23.8013$

$Y = 1.91E-08X + 5.23E-05$

$K = 1/(B \cdot A_0) = 2.20E+06$ (binding constant for Hg(II))

$SD = 1/(SD \cdot A_0) = \pm 82.9$

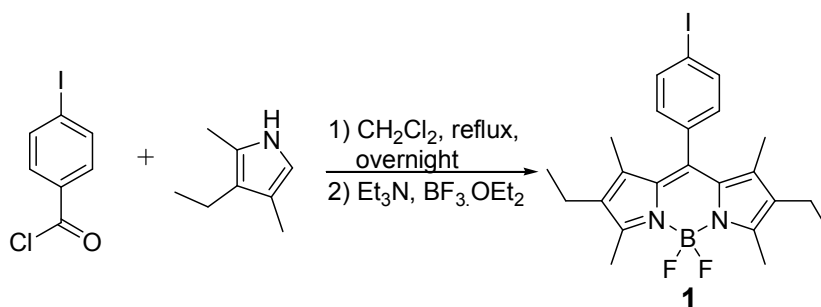
	Value	Error		
A	5.23E-05	5.56E-04		
B	1.91E-08	1.79E-09		
R	SD	N	P	
<hr/>				
	0.98702	5.07E-04	5	0.00177

Experimental Section

Materials and General Instrumentation. All chemicals and solvents purchased from Aldrich were used without further purification. ^1H -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. HRMS (ESI) measurements were done at the Mass Spec & Proteomics Facility, Ohio State University, Columbus, OH, USA.

Numbering of the chemical compounds in the following section is not the same as the main text and based on the sequence of synthetic procedures.

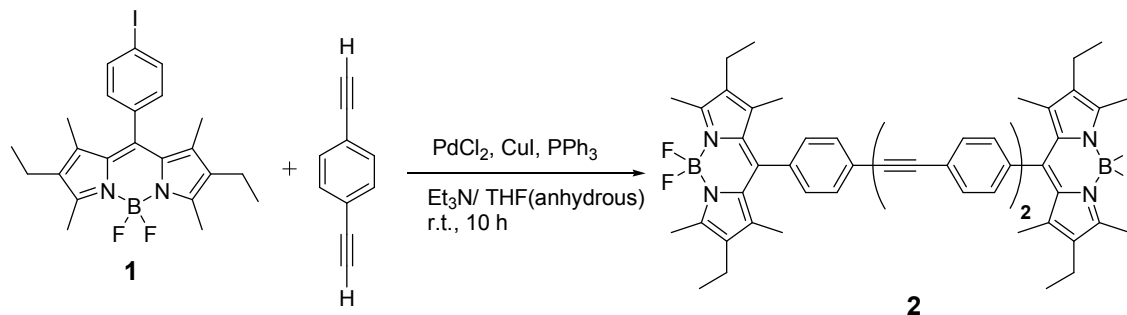
2,6-diethyl-4,4-difluoro-1,3,5,7-tetramethyl-8-(4-iodophenyl)-4-bora-3a,4a-diaza-s-inadcene 1: 4-iodobenzoylchloride (7.7 mmol, 2.05 g) and 3-ethyl-2,4-dimethyl pyrrole (15.4 mmol, 1.9 g) were dissolved in CH₂Cl₂ and refluxed for 3 h. After 3 h, Et₃N (5 ml) and BF₃.OEt₂ (5 ml) were added. Then bright yellowish fluorescence was observed. Crude product washed three times with water and dried over Na₂SO₄ and concentrated in *vacuo*. Then the crude product purified by silica gel column chromatography (eluent CHCl₃). First fraction which has bright yellow fluorescence was collected. Orange solid (2.53 g, 65 %).[ref. SR1]



Boradiazaindacene dimer 2; Compound **1** (1.98 mmol, 1 g), 1,4-diethynylbenzene (0.988 mmol, 0.125 g), PdCl₂ (0.15 mmol, 27 mg), CuI (0.30 mmol, 57 mg) and PPh₃ (0.60 mmol, 157 mg) were added to the round bottomed flask which was previously flushed with Argon. As a solvent Et₃N (5 ml) and anhydrous THF (50 ml) was added. The reaction mixture was stirred overnight at r.t.. After completion of the reaction, solvent was removed in *vacuo*. The crude product purified by silica gel column chromatography (eluent first; CHCl₃ then 2 CHCl₃ : 1 Hexane). Orange solid (724 mg, % 83) .

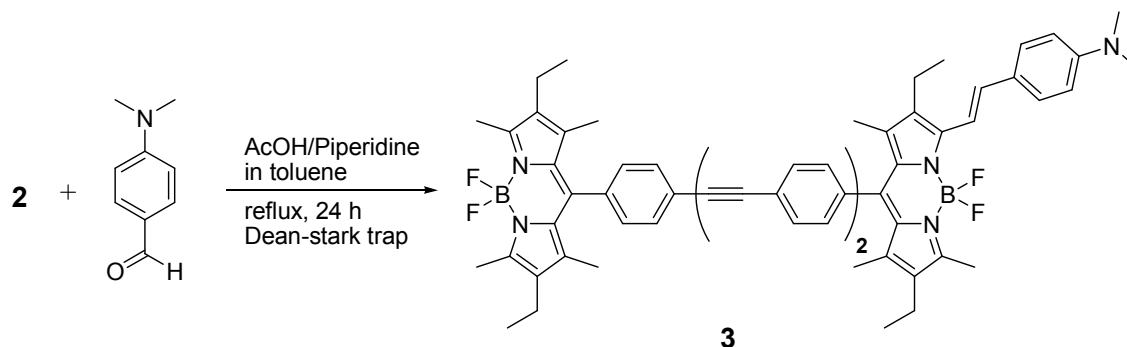
¹H NMR (400 MHz, CDCl₃) δ; 7.60 (d, *J*=8.10 Hz, 4H), 7.50 (s, 4H), 7.24 (d, *J*=8.10 Hz, 4H), 2.47 (s, 12H), 2.24 (q, *J*=7.50 Hz, 8H), 1.27 (s, 12H), 0.92 (t, *J*=7.50 Hz, 12H) ; ¹³C

NMR (100 MHz, CDCl₃) δ : 154.1, 139.2, 138.2, 136.2, 132.9, 132.3, 131.7, 130.6, 128.7, 123.6, 123.1, 90.8, 90.3, 17.1, 14.6, 12.5, 11.9 ; ESI-HRMS calcd for [M+Na] 905.4525 found. 905.4495 Δ =3.3 ppm.



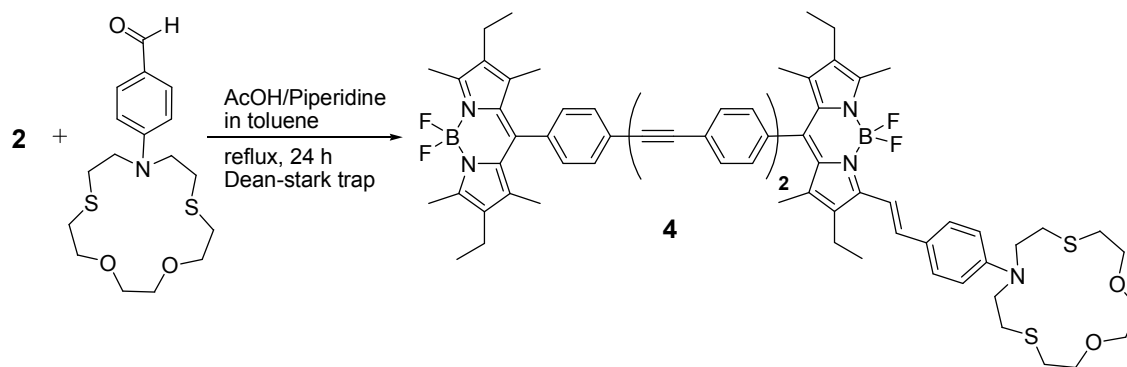
***p*-Dimethylaminostyryl modified boradiazaindacene dimer 3**; Compound **2** (0.283 mmol, 250 mg) and *p*-dimethylaminobenzaldehyde (0.283 mmol, 43 mg) were refluxed in a mixture of toluene (75 ml), glacial acetic acid (220 μ l), piperidine (260 μ l). Any water formed during the reaction, was removed azeotropically by heating overnight in a Dean-Stark apparatus. The solvent was removed in *vacuo*, then crude product purified by silica gel column chromatography (eluent CHCl₃). The blue colored fraction was collected (106 mg, % 37).

¹H NMR (400 MHz, CDCl₃) δ : 7.60 (d, J =7.95 Hz, 4H), 7.56-7.46 (m, 7H), 7.27-7.23 (m, 4H), 7.14 (d, J =16.70 Hz, 1H), 6.68 (br, 2H), 2.96 (s, 6H), 2.57-2.47 (m, 11H), 2.30-2.21 (m, 6H), 1.30 (s, 3H), 1.27 (s, 9H), 1.09 (t, J =7.55 Hz, 3H), 0.95-0.90 (m, 9H) ; ¹³C NMR (100 MHz, CDCl₃) δ : 153.1, 138.2, 137.2, 136.3, 135.5, 135.1, 132.3, 131.9, 131.2, 131.1, 130.6, 129.5, 127.9, 127.7, 127.6, 122.6, 122.5, 122.1, 122.0, 89.9, 89.8, 89.2, 40.4, 17.4, 16.1, 13.6, 13.0, 11.7, 11.5, 10.8, 10.6 ; ESI-HRMS calcd for [M+Na] 1036.5260 found 1036.5281 Δ =2.0 ppm.



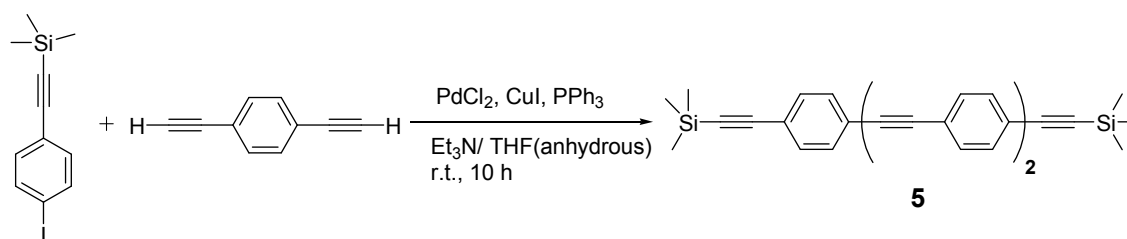
Chemosensor 4: Compound **2** (0.164 mmol, 145 mg) and 4-(1-aza-7,10-dioxo-4,13-dithiacyclopentadecyl)benzaldehyde (synthesized according to literature procedure, starting from 3-N-phenyl-3-aza-1,5-pentanediol [SR2]; 0.18 mmol, 64 mg) were dissolved in toluene (60 ml), glacial acetic acid (130 μ l), piperidine (153 μ l). Any water formed during the reaction, was removed azeotropically by heating overnight in a Dean-Stark apparatus. Solvent was removed under reduced pressure and the crude product first purified by silica gel column chromatography (eluent 99 CHCl₃ : 1 MeOH), then further purified by Preparative thin layer chromatography (PTLC) in the same solvent system. The blue fraction was isolated (82 mg, % 41).

¹H NMR (400 MHz, CDCl₃) δ : 7.60 (d, J =7.90 Hz, 4H), 7.53-7.43 (m, 7H), 7.27-7.23 (m, 4H), 7.12 (d, J =16.55 Hz, 1H), 6.65 (br, 2H), 3.74 (t, J =5.04 Hz, 4H), 3.65-3.59 (m, 8H), 2.88-2.84 (m, 4H), 2.70 (t, J =5.04 Hz, 4H), 2.56-2.47 (m, 11H), 2.25-2.17 (m, 6H), 1.29 (s, 3H), 1.27 (s, 9H), 1.06 (t, J =7.40 Hz, 3H), 0.92 (t, J =7.40 Hz, 9H); ¹³C NMR (100 MHz, CDCl₃) δ : 154.1, 139.2, 138.2, 136.1, 133.3, 132.9, 132.2, 131.6, 130.5, 129.0, 128.9, 128.6, 123.5, 123.1, 90.9, 90.1, 74.2, 70.7, 51.1, 31.9, 31.5, 29.6, 29.2, 21.0, 17.1, 14.5, 14.1, 14.0, 12.5, 11.8, 11.6; ESI-HRMS calcd for [M+Na] 1242.5695 found 1242.5710 Δ =1.2 ppm.

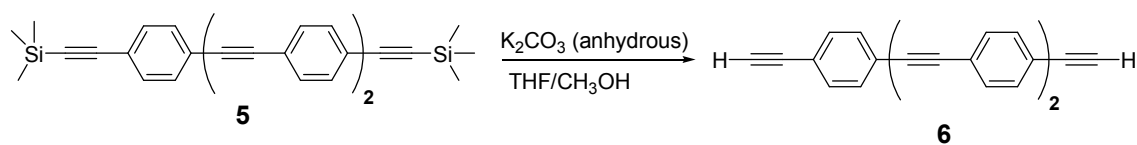


TMS-protected spacer 5: (2-(4-iodophenyl)ethynyl)trimethylsilane (synthesized acc. to literature procedure [SR3]; 5.5 mmol, 1.65 g), 1,4-diethynylbenzene (2.75 mmol, 0.35 g), PdCl_2 (0.275 mmol, 49 mg), CuI (0.55 mmol, 105 mg) and PPh_3 (1.1 mmol, 289 mg) were added to the round bottomed flask which was previously flushed with Argon. As a solvent Et_3N (5 ml) and anhydrous THF (50 ml) was added. The reaction mixture was stirred overnight at r.t. under Ar atmosphere. After completion of the reaction, solvent was removed in *vacuo*. The crude product first purified by silica gel column chromatography (eluent CHCl_3), then further purified by washing yellowish solid with n-hexane to yield white solid. White solid (1.13 g, % 87) .

^1H NMR (400 MHz, CDCl_3) δ : 7.43 (s, 4H), 7.39 (s, 8H), 0.19 (s, 18H).

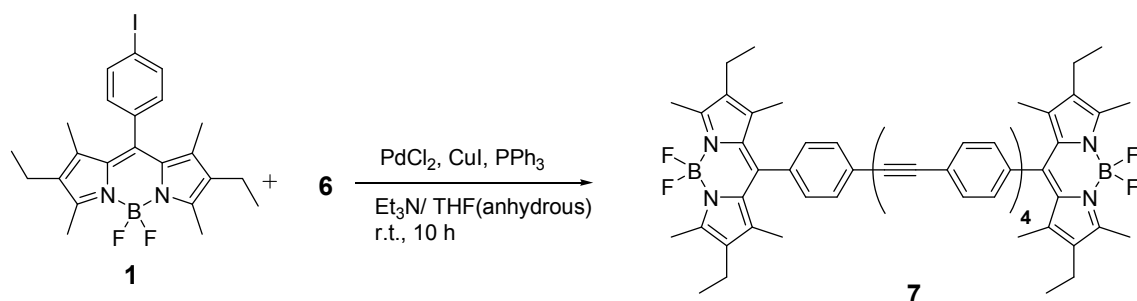


Deprotection of compound 5: Compound **5** (1.02 mmol, 480 mg) and anhydrous K_2CO_3 (6.12 mmol, 845 mg) was added to THF/MeOH mixture (50/50 ml) the mixture was stirred for 6h at rt. After completion of the reaction, solvents were removed in *vacuo*. Then the crude product washed with water and dried under vacuum. The yellowish solid was used through next step without further purification.



Boradiazaindacene dimer 7: Compound **2** (0.9 mmol, 456 mg), Compound **6** (0.43 mmol, 140 mg), PdCl₂ (0.086 mmol, 16 mg), CuI (0.18 mmol, 34 mg) and PPh₃ (0.36 mmol, 95 mg) were added to the round bottomed flask which was previously flushed with Argon. As a solvent Et₃N (5 ml) and anhydrous THF (50 ml) was added. The reaction mixture was stirred overnight at r.t.. After completion of the reaction, solvent was removed in *vacuo*. The crude product purified by silica gel column chromatography (eluent CHCl₃). Orange solid (354 mg, % 76) .

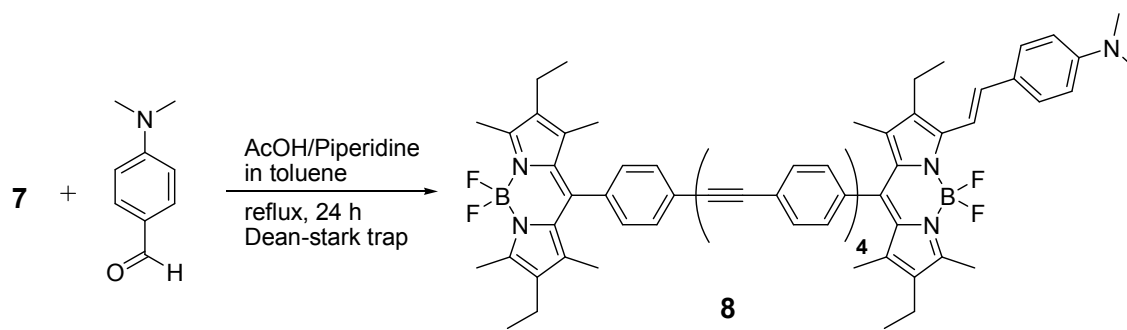
¹H NMR (400 MHz, CDCl₃) δ; 7.59 (d, *J*=8.10 Hz, 4H), 7.48 (s, 8H), 7.46 (s, 4H), 7.24 (d, *J*=8.10 Hz, 4H), 2.47 (s, 12H), 2.24 (q, *J*=7.40 Hz, 8H), 1.27 (s, 12H), 0.92 (t, *J*=7.40 Hz, 12H) ; ¹³C NMR (100 MHz, CDCl₃) δ; 154.1, 139.2, 138.2, 136.1, 132.9, 132.3, 132.2, 131.6, 130.5, 128.6, 128.4, 123.6, 123.2, 123.1, 122.9, 91.2, 91.0, 90.8, 90.3, 17.1, 14.6, 12.5, 11.8 ; ESI-HRMS calcd for [M+Na] 1105.5151 found 1105.5144 Δ=0.6 ppm.



***p*-Dimethylaminostyryl modified boradiazaindacene dimer 8:** Compound **7** (0.092 mmol, 100 mg) and *p*-dimethylaminobenzaldehyde (0.100 mmol, 15 mg) were refluxed in a mixture of toluene (50 ml), glacial acetic acid (75 μl), piperidine (85 μl). Any water formed

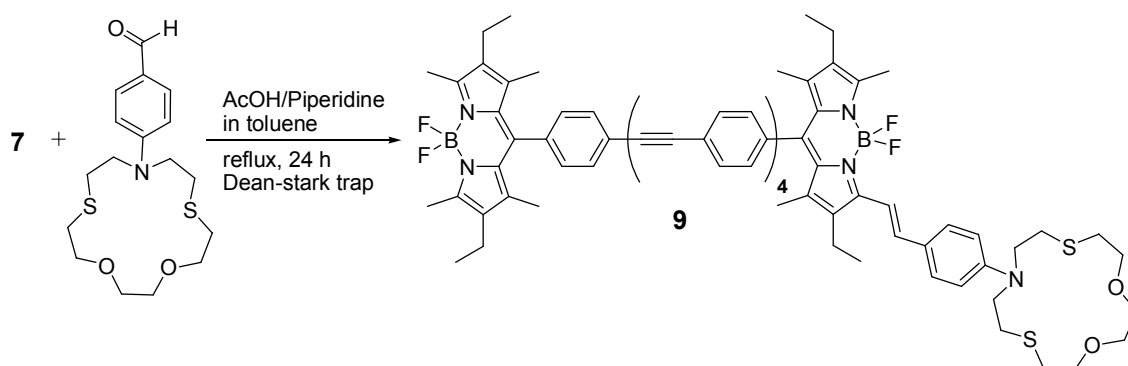
during the reaction, was removed azeotropically by heating overnight in a Dean-Stark apparatus. The solvent was removed in *vacuo*, then crude product purified by silica gel column chromatography (eluent first CHCl₃, then CHCl₃:n-hexane, 2:1, v:v). The blue colored fraction was collected (36 mg, % 32).

¹H NMR (400 MHz, CDCl₃) δ : 7.63-7.56 (m, 5H), 7.52-7.46 (m, 14H), 7.27-7.23 (m, 4H), 7.14 (d, J =16.70 Hz, 1H), 6.83 (br, 2H), 2.98 (s, 6H), 2.55-2.47 (m, 11H), 2.27-2.21 (m, 6H), 1.3 (s, 3H), 1.27 (s, 9H), 1.06 (t, J =7.55 Hz, 3H), 0.95-0.90 (m, 9H); ESI-HRMS calcd for 1213.5988 found 1213.6049 Δ =5.0 ppm



Chemosensor 9: Compound 7 (0.096 mmol, 104 mg) and 4-(1-aza-7,10-dioxa-4,13-dithiacyclopentadecyl)benzaldehyde (synthesized according to literature procedure, starting from 3-N-phenyl-3-aza-1,5-pentanediol [SR2]; 0.105 mmol, 38 mg) were dissolved in toluene (40 ml), glacial acetic acid (75 μ l), piperidine (90 μ l). Any water formed during the reaction, was removed azeotropically by heating overnight in a Dean-Stark apparatus. Solvent was removed under reduced pressure and the crude product first purified by silica gel column chromatography (eluent 99 CHCl₃ : 1 MeOH), then further purified by Preparative thin layer chromatography (PTLC) in the same solvent system. The blue fraction was isolated (65 mg, % 48).

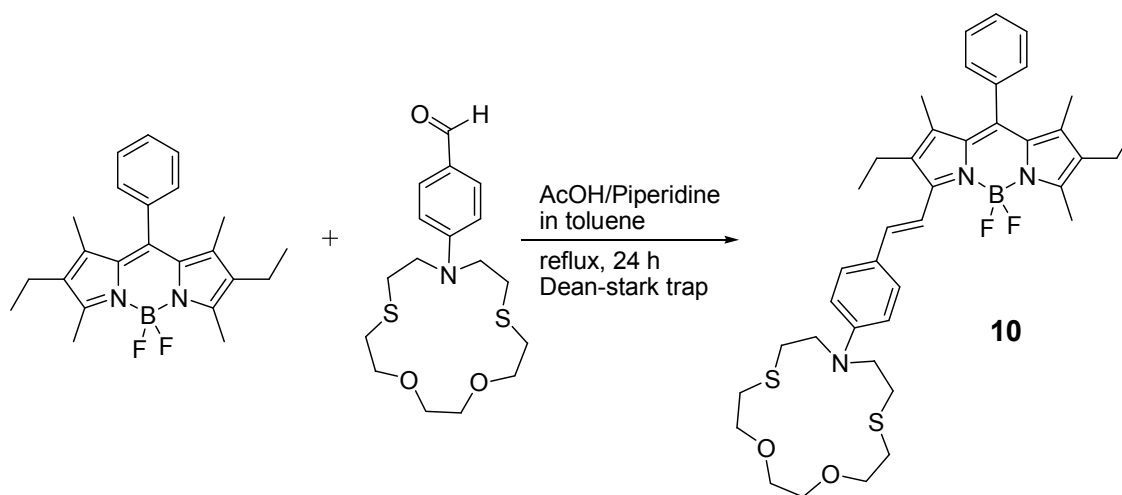
^1H NMR (400 MHz, CDCl_3) δ : 7.59 (d, $J=8.05$ Hz, 4H), 7.52-7.42 (m, 15H), 7.26-7.22 (m, 4H), 7.12 (d, $J=16.50$ Hz, 1H), 6.61 (br, 2H), 3.74 (t, $J=4.90$ Hz, 4H), 3.64-3.58 (m, 8H), 2.85 (m, 4H), 2.69 (t, $J=4.90$ Hz, 4H), 2.54-2.46 (m, 11H), 2.25-2.19 (m, 6H), 1.29 (s, 3H), 1.26 (s, 9H), 1.07 (t, $J=7.60$ Hz, 3H), 0.92 (t, $J=7.60$ Hz, 9H); ^{13}C NMR (100 MHz, CDCl_3) δ : 154.1, 139.2, 138.5, 138.2, 137.4, 136.5, 136.1, 133.3, 132.9, 132.5, 132.2, 131.6, 131.2, 130.5, 129.1, 128.9, 128.6, 123.6, 123.5, 123.2, 123.1, 123.0, 122.9, 91.1, 90.8, 90.7, 90.3, 74.2, 70.8, 52.4, 31.9, 31.4, 29.7, 29.4, 22.7, 18.4, 17.1, 14.6, 14.0, 12.5, 11.9, 11.6 ; ESI-HRMS calcd for $[\text{M} + \text{Na} + 3\text{H} - 2\text{BF}_2]$ 1347.6434 found. 1347.9

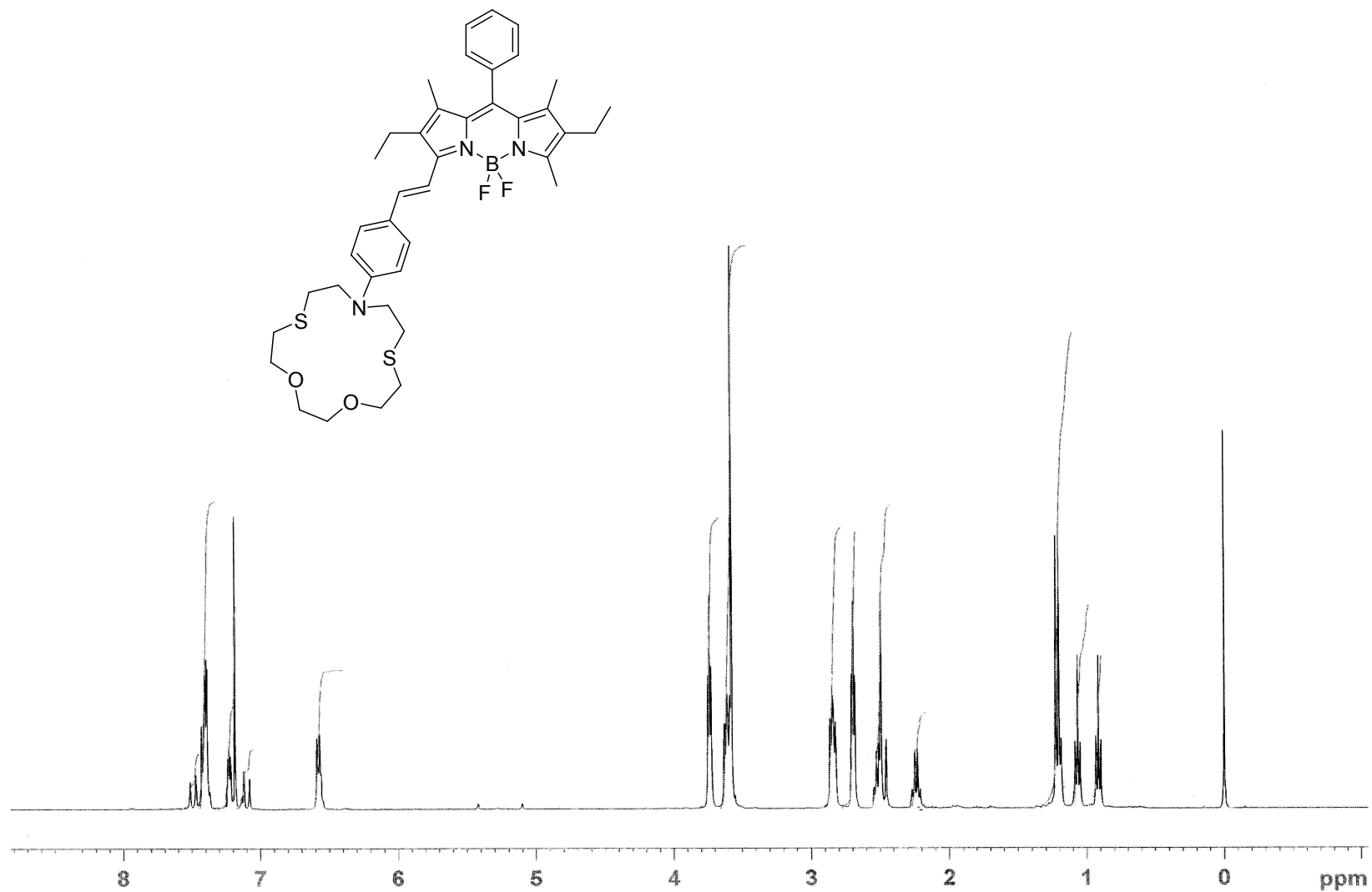


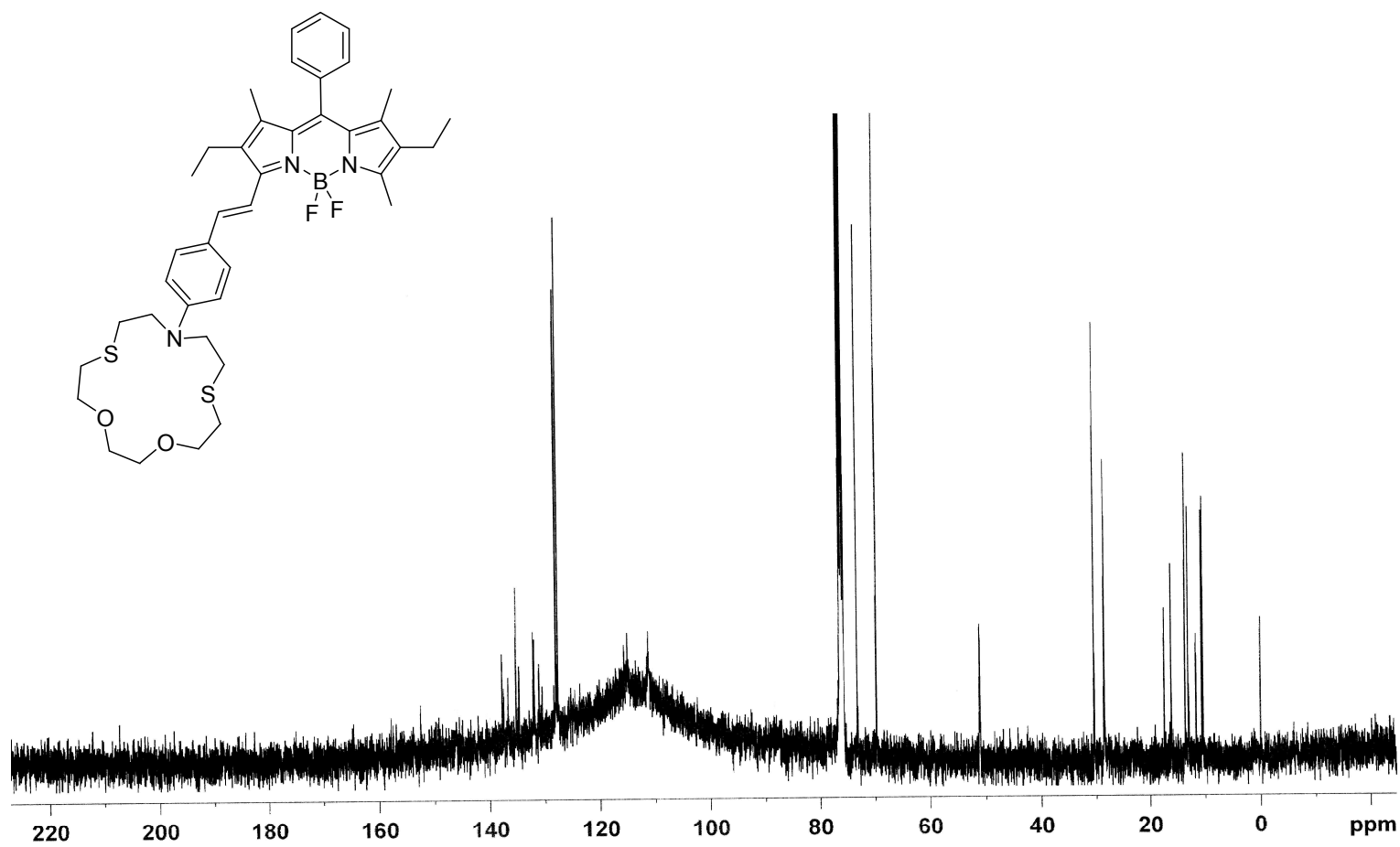
Reference compound 10: 4,4-difluoro-8-phenyl-1,3,5,7-tetramethyl-2,6-diethyl-4-bora-3a,4a-diaza-s-indacene (0.263 mmol, 100 mg) and 4-(1-aza-7,10-dioxa-4,13-dithiacyclopentadecyl)benzaldehyde (synthesized according to literature procedure, starting from 3-N-phenyl-3-aza-1,5-pentanediol [SR2]; 0.265 mmol, 95 mg) were dissolved in toluene (50 ml), glacial acetic acid (165 μl), piperidine (190 μl). Any water formed during the reaction, was removed azeotropically by heating overnight in a Dean-Stark apparatus. Solvent was removed under reduced pressure and the crude product purified by silica gel column chromatography (eluent 99 CHCl_3 : 1 MeOH). The blue fraction was isolated (106 mg, % 56).

^1H NMR (400 MHz, CDCl_3) δ : 7.49 (d, $J=16.65$ Hz, 1H), 7.43-7.37 (m, 5H), 7.24-7.21 (m, 2H), 7.10 (d, $J=16.65$ Hz, 1H), 6.58 (m, 2H), 3.74 (t, $J=4.70$ Hz, 4H), 3.63-3.58 (m, 8H), 2.84

(m, 4H), 2.69 (t, $J=4.70$ Hz, 4H), 2.52-2.45 (m, 5H), 2.22 (q, $J=7.50$ Hz, 2H), 1.23 (s, 3H), 1.19 (s, 3H), 1.07 (t, $J=7.50$ Hz, 3H), 0.92 (t, $J=7.50$ Hz, 3H); ^{13}C NMR (100 MHz, CDCl_3) δ ; 153.2, 137.7, 137.4, 136.6, 135.2, 134.7, 132.1, 131.8, 131.0, 130.5, 128.0, 127.9, 127.6, 125.3, 115.6, 114.9, 111.7, 73.2, 69.71, 51.1, 30.4, 28.6, 17.4, 16.3, 13.6, 13.0, 11.6, 10.6, 10.3; ESI-HRMS calcd for $[\text{M}+\text{Na}]$ 740.3303 found 740.3325 $\Delta=3.0$ ppm.







11117 AC-X1L Akkaya/METU

LCT electrospray

L080906K 77 (1.430) AM (Cen,4, 80.00, Ar,5000.0,0.00,1.00); Sm (SG, 5x3.00); Cm (77:95)

Mass Spec & Proteomics Facility

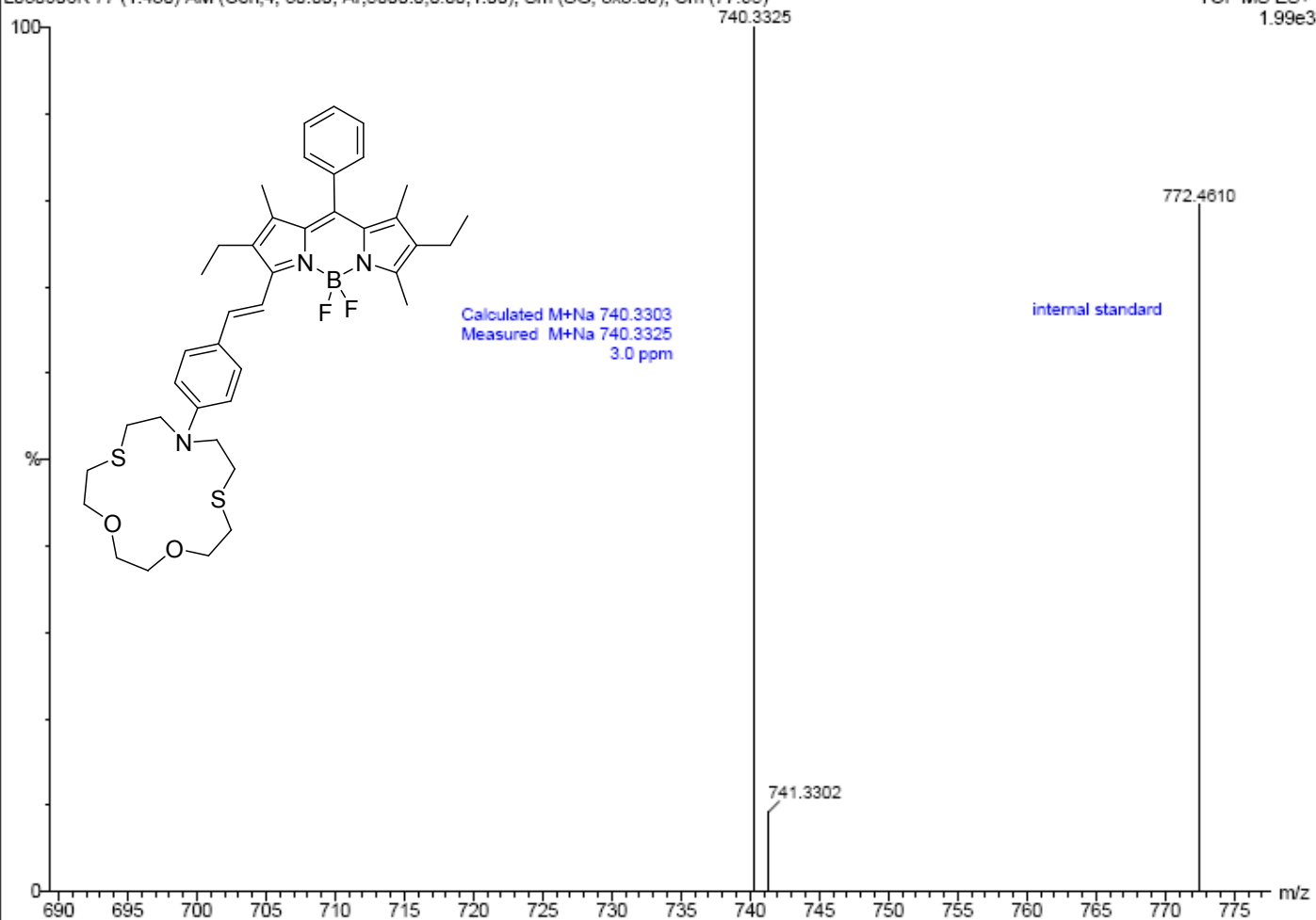
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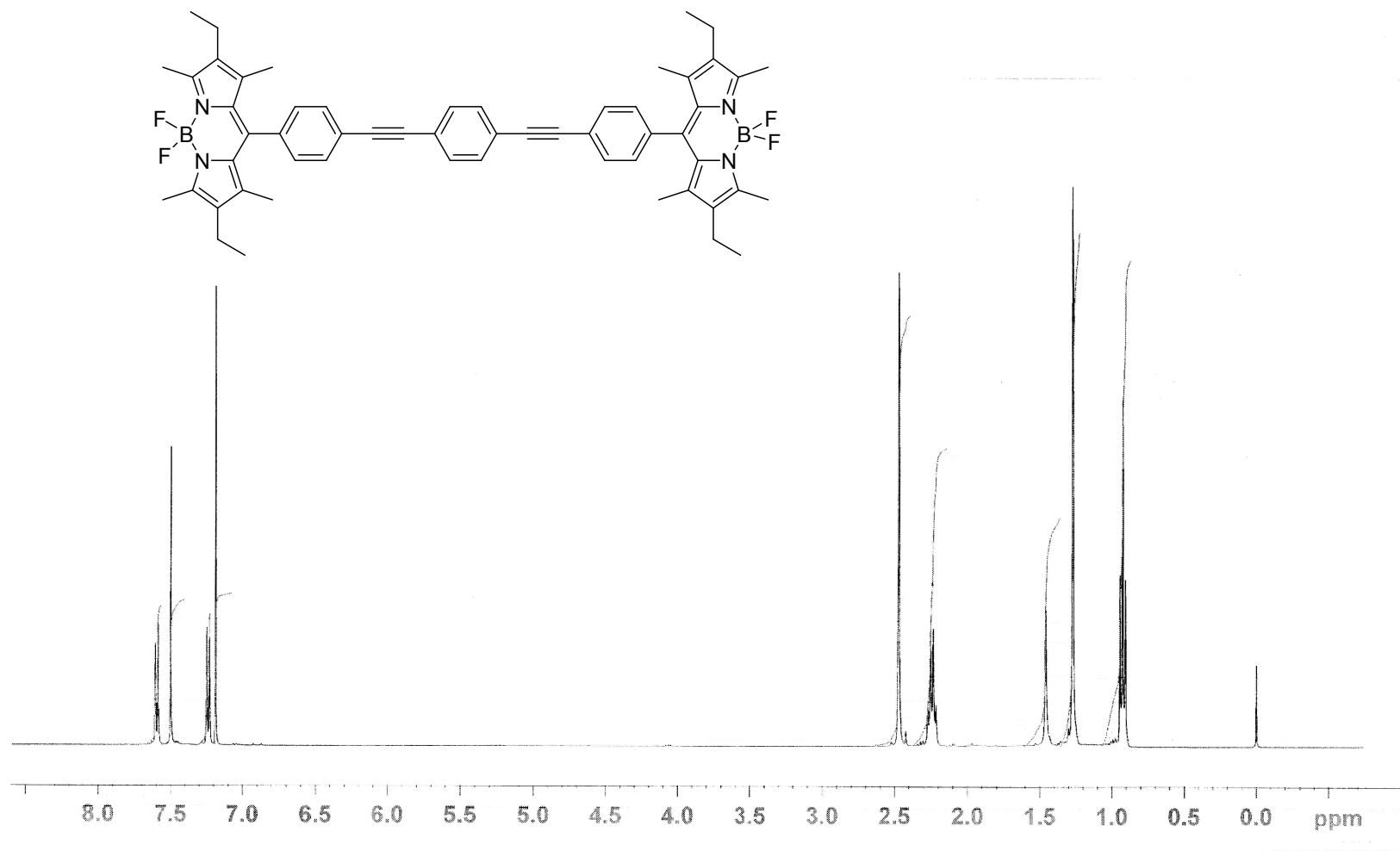
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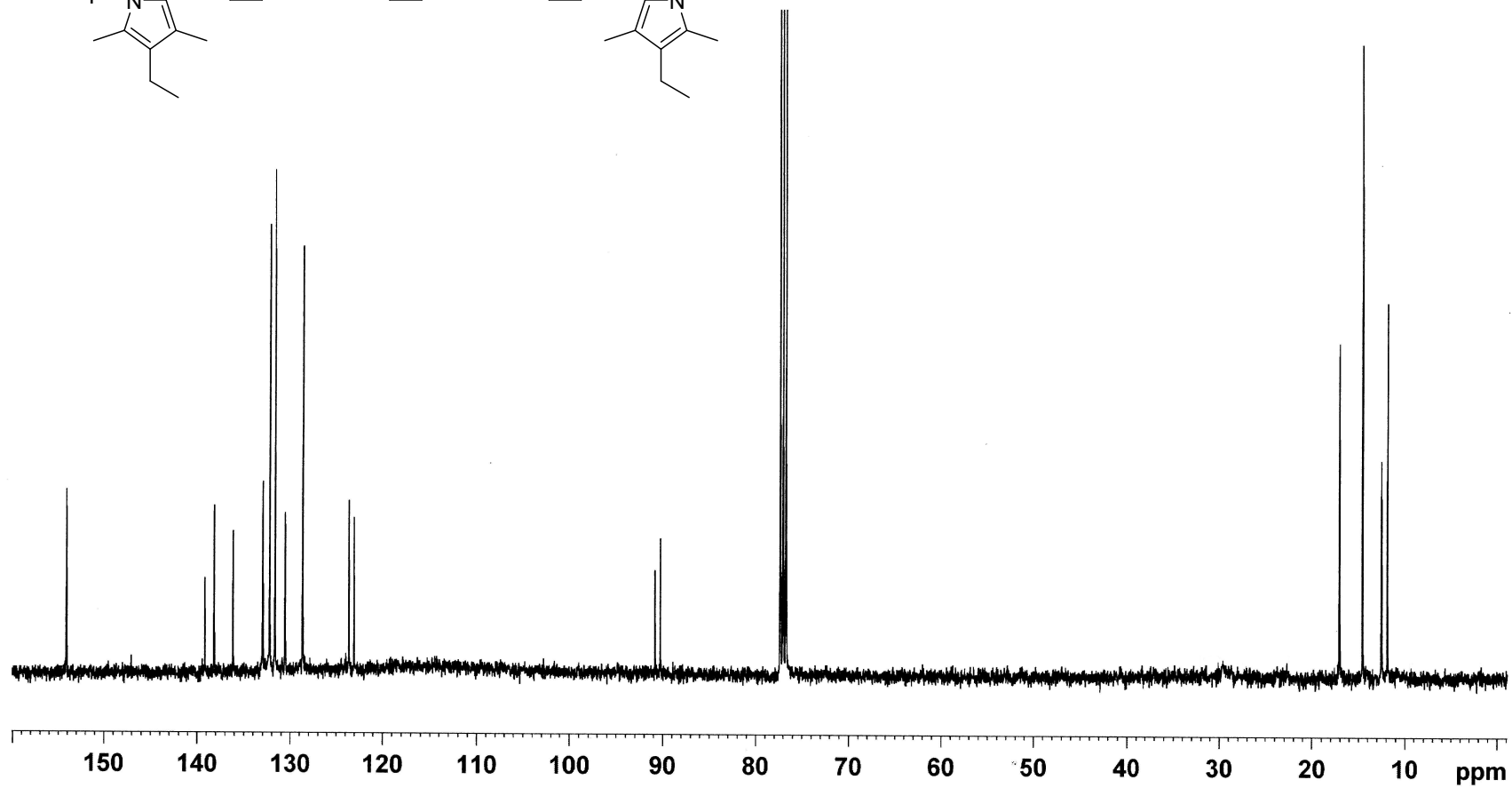
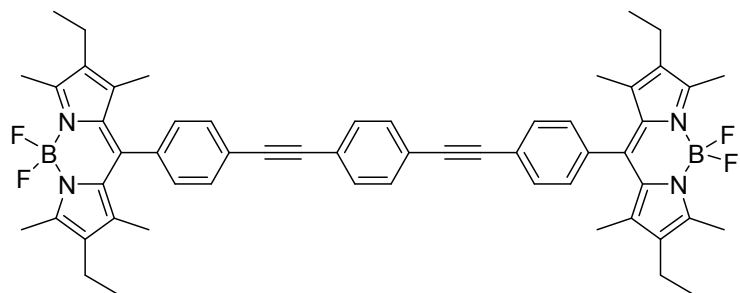
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TOF MS ES+

1.99e3







11117 AC-X3 Akkaya/METU

Mass Spec & Proteomics Facility

09-Aug-2006

LCT electrospray

(614) 292-4821

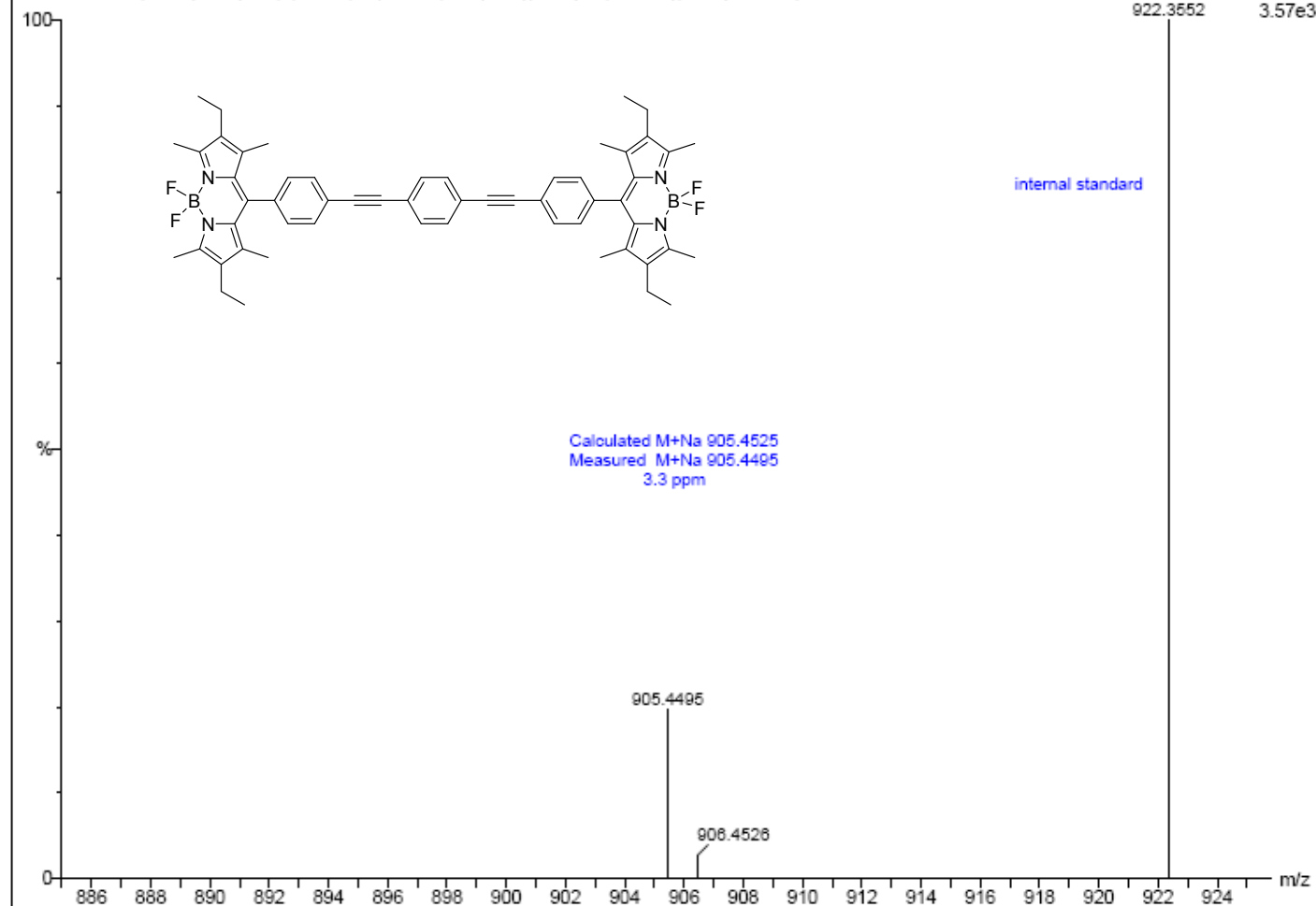
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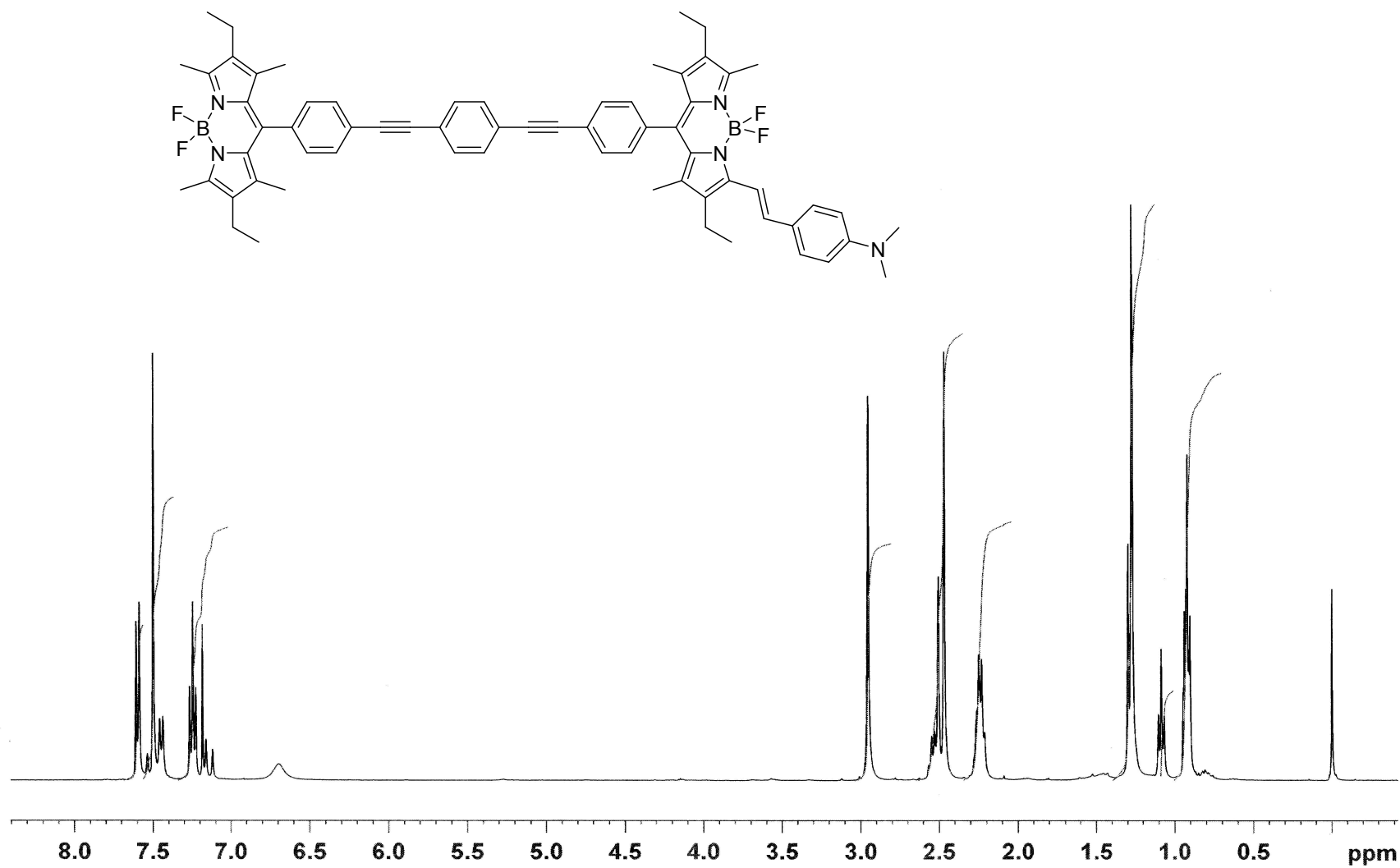
L080906J 110 (2.017) AM (Cen,4, 80.00, Ar,5000.0,0.00,1.00); Sm (SG, 5x3.00); Cm (108:120)

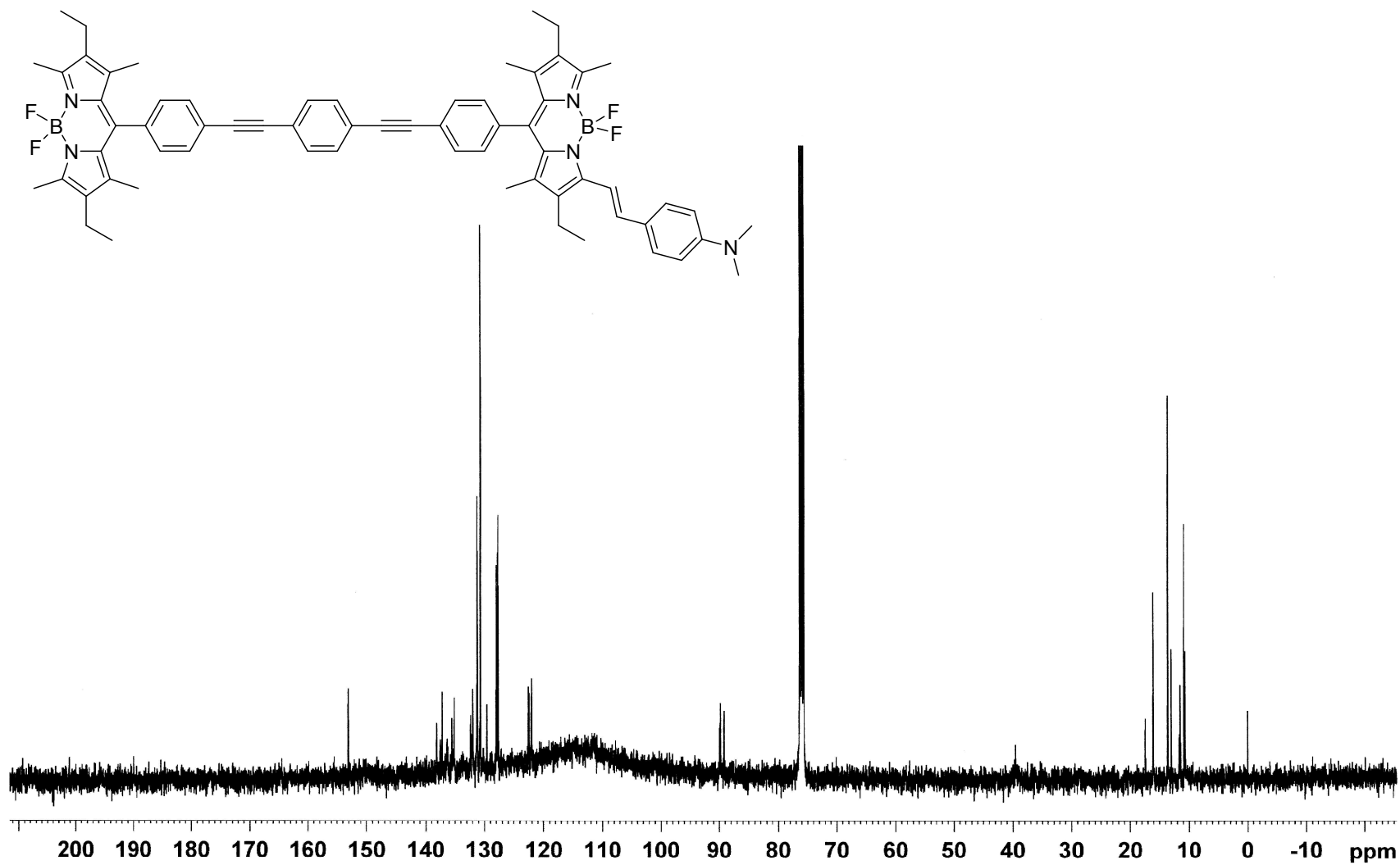
TOF MS ES+

922.3552

3.57e3







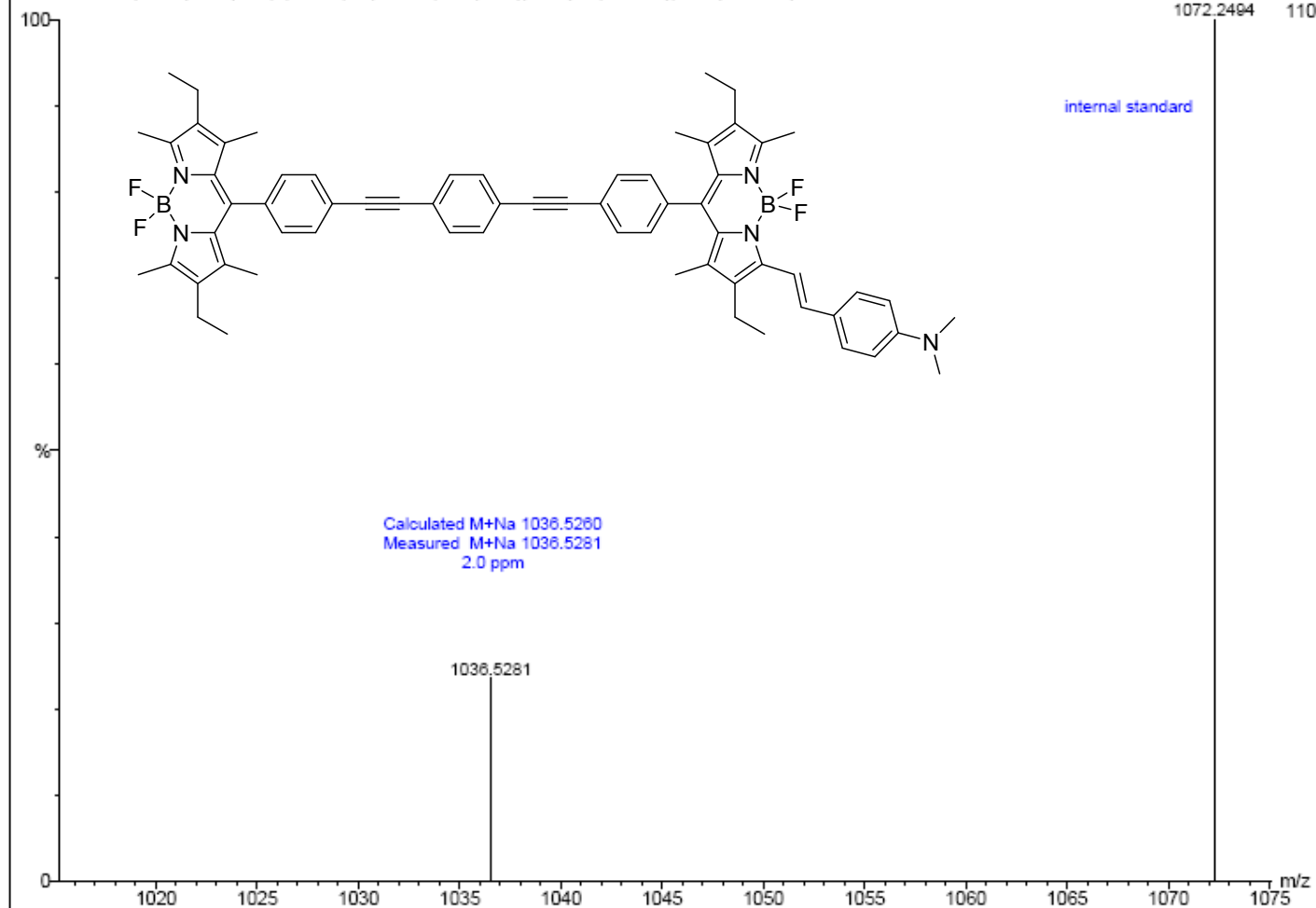
11117 AC-X3DMA Akkaya/METU
LCT electrospray

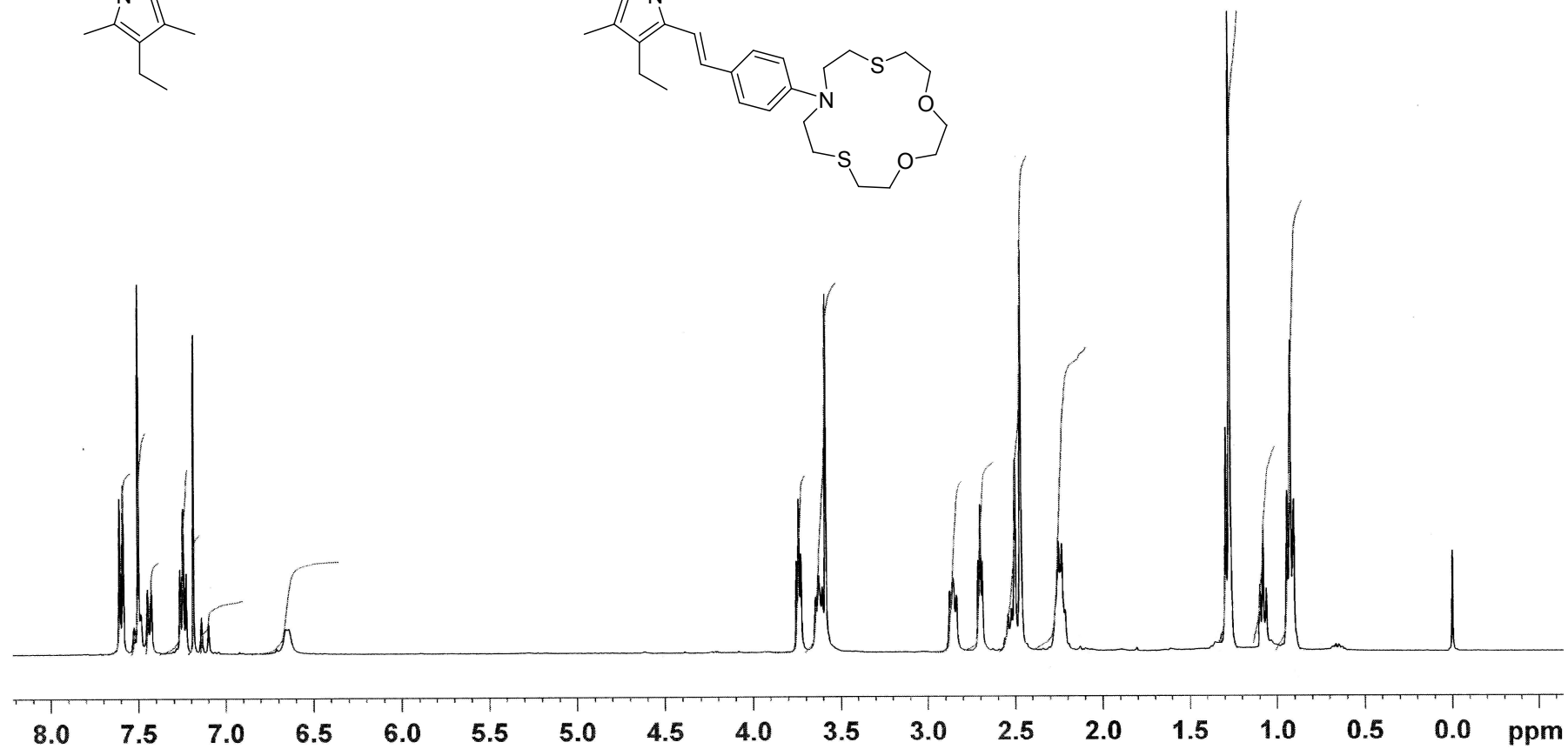
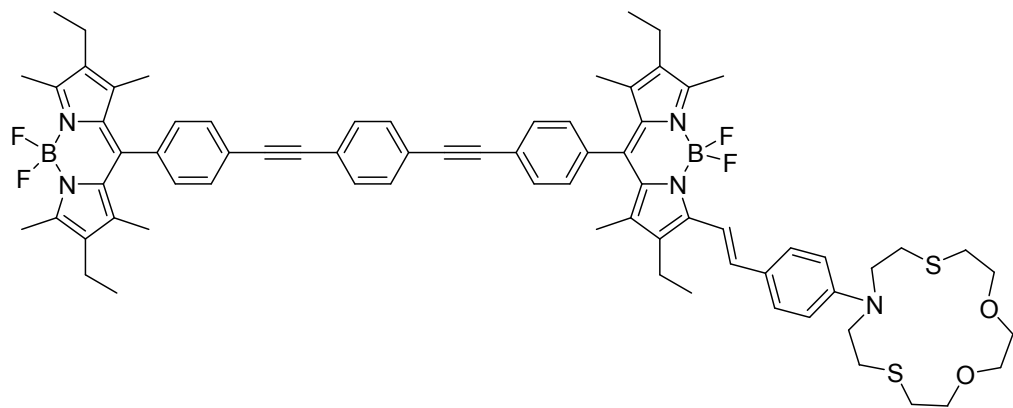
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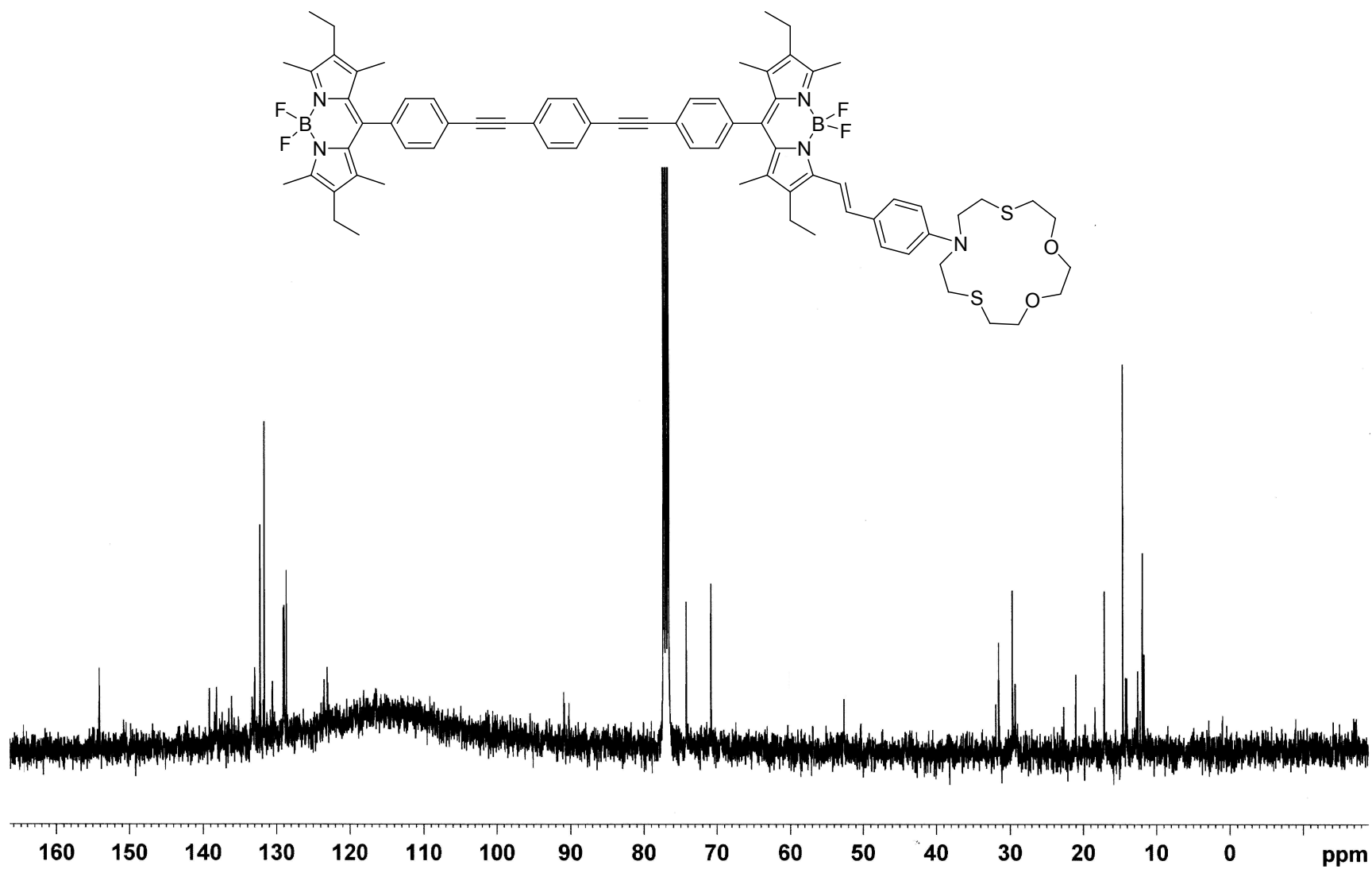
09-Aug-2006
www.ccic.ohio-state.edu

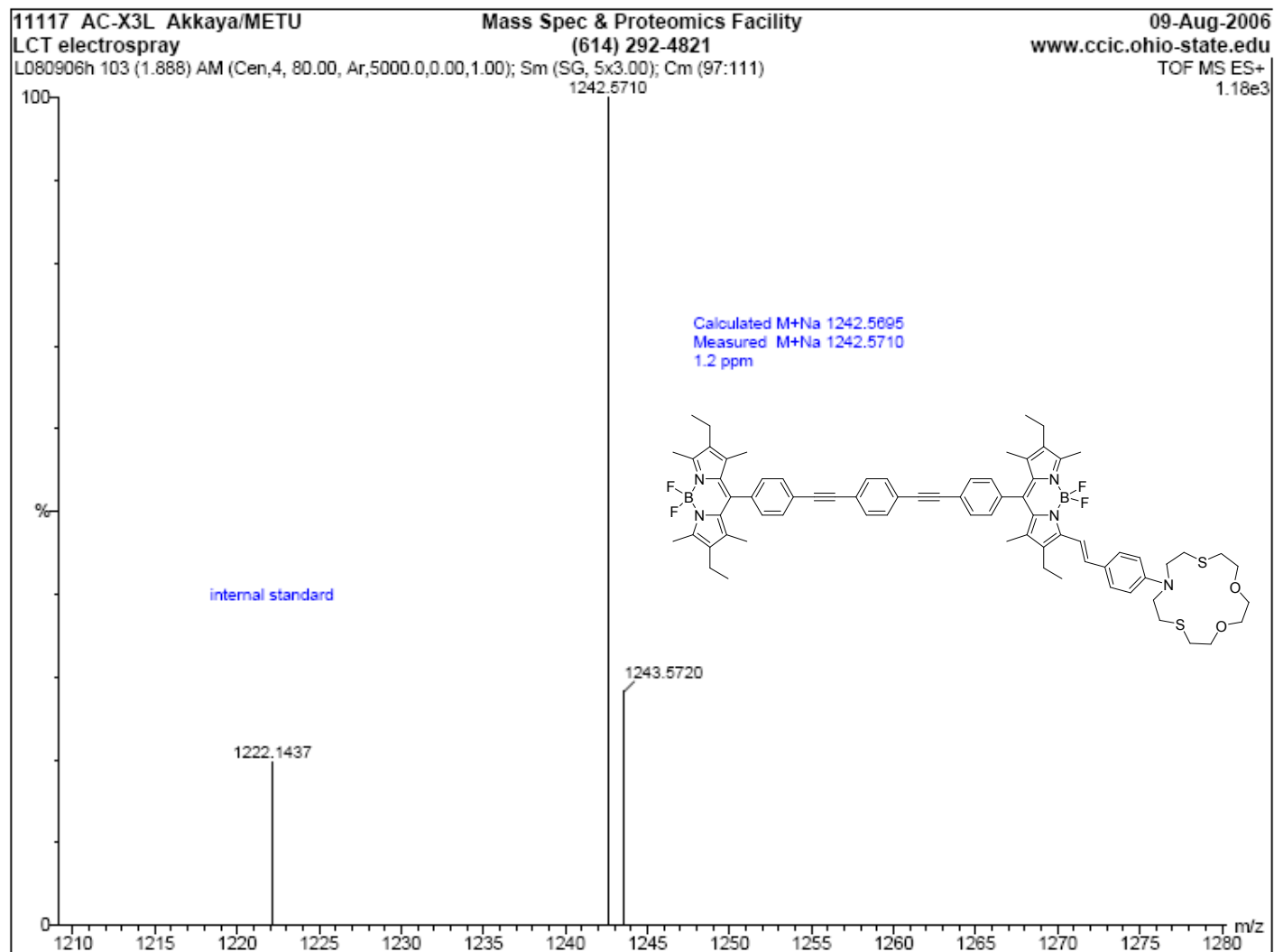
L080906i 123 (2.255) AM (Cen,4, 80.00, Ar,5000.0,0.00,1.00); Sm (SG, 5x3.00); Cm (122:127)

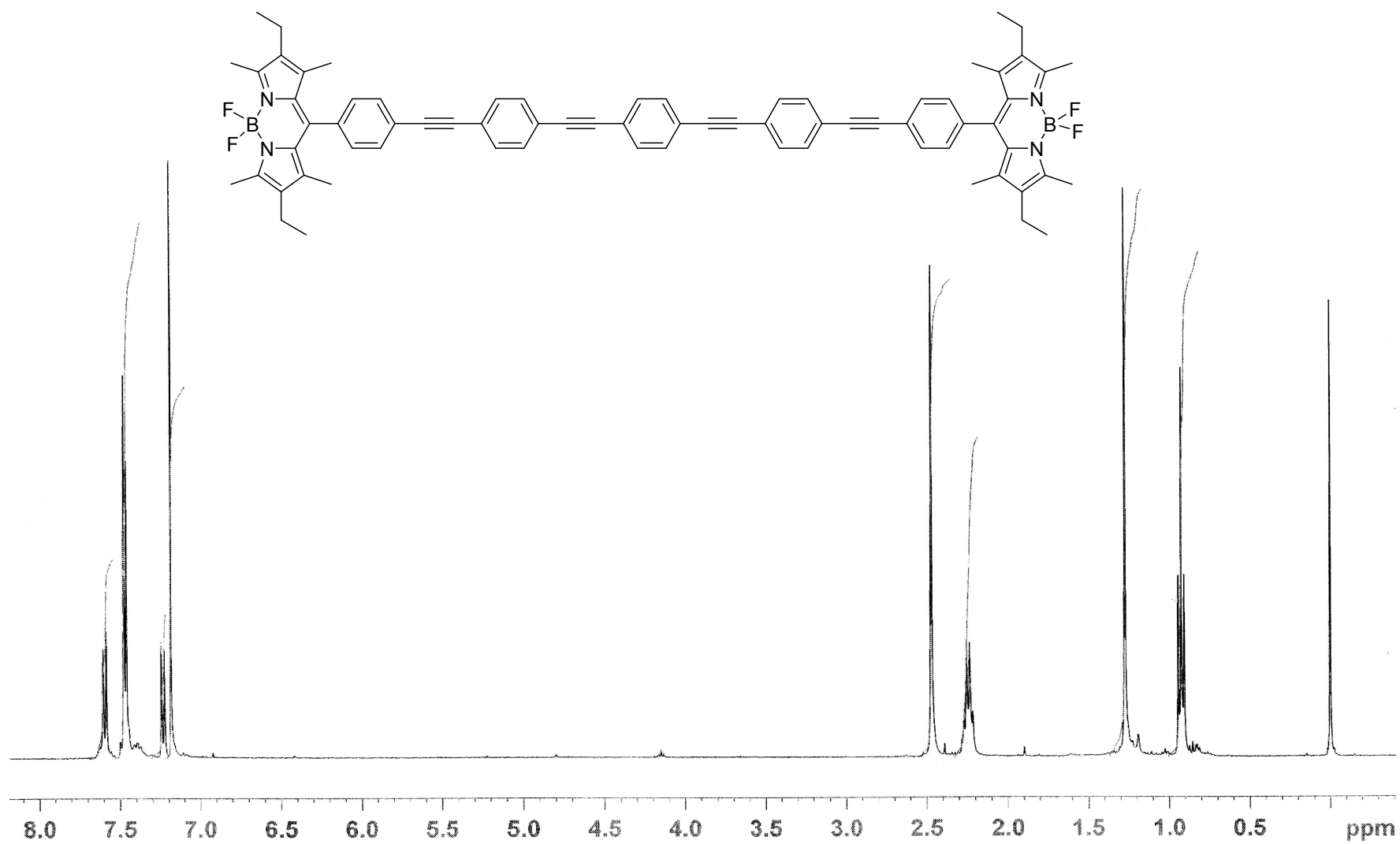
TOF MS ES+
1072.2494 110

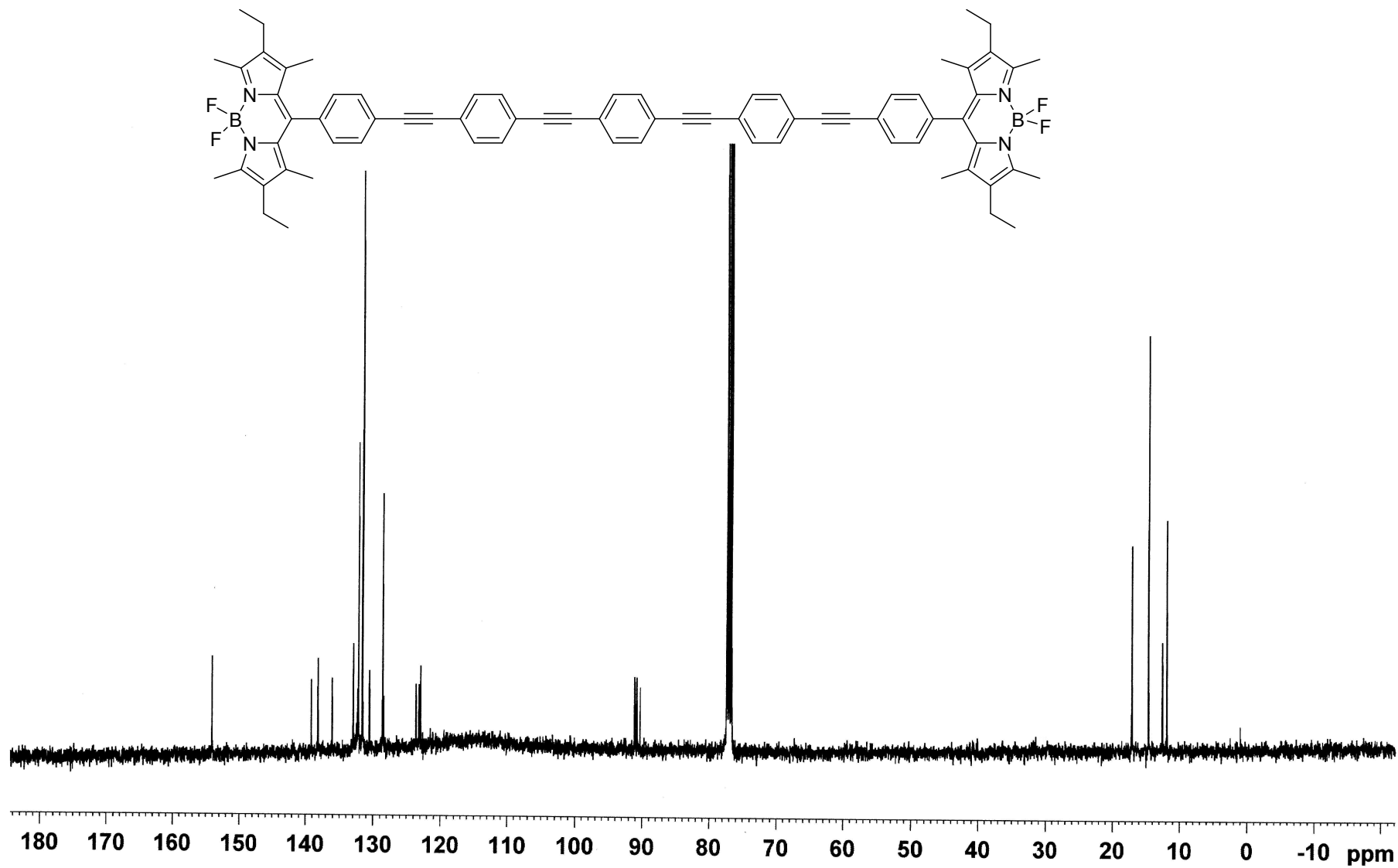












11117 AC-X5 Akkaya/METU

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LCT electrospray

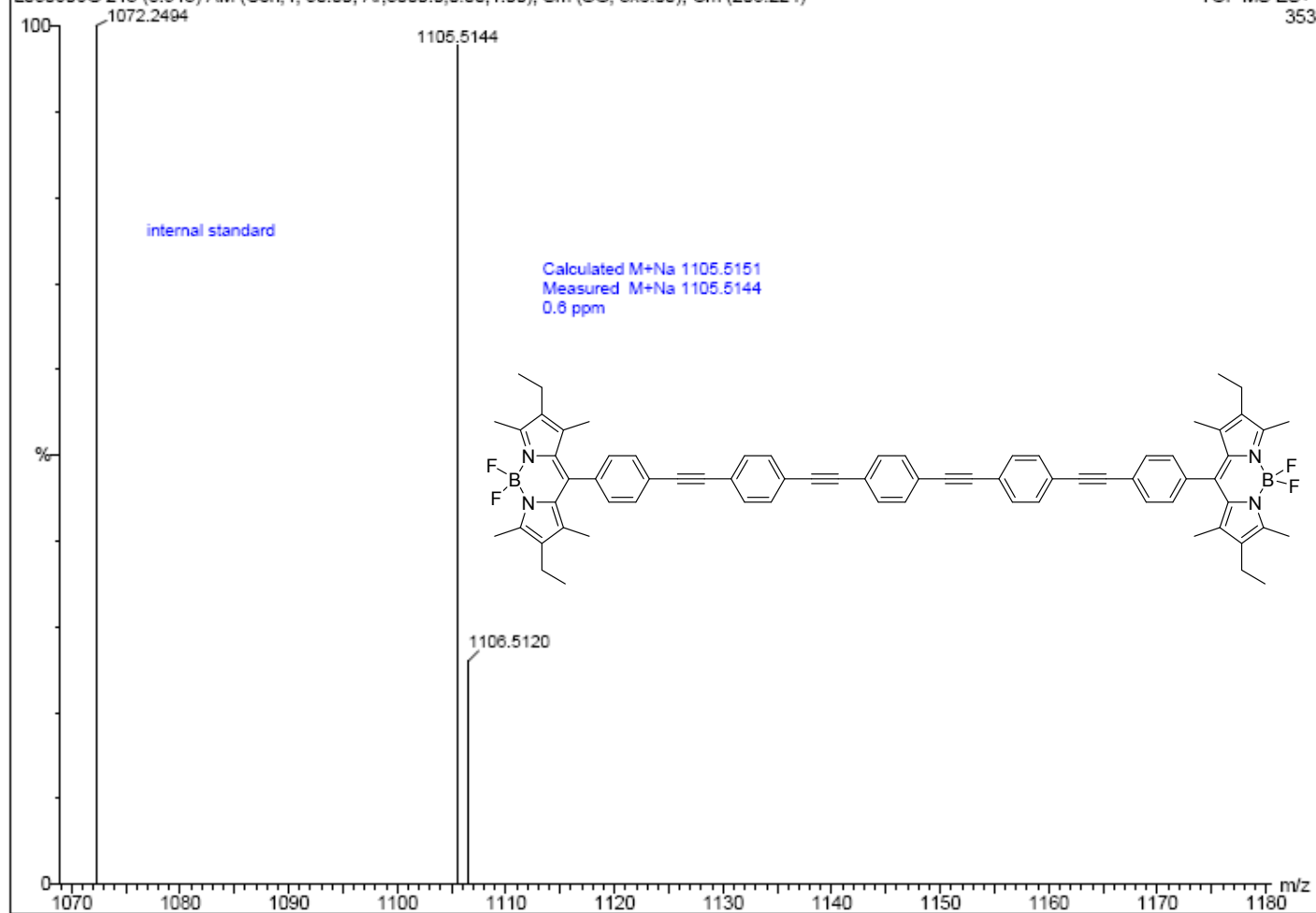
(614) 292-4821

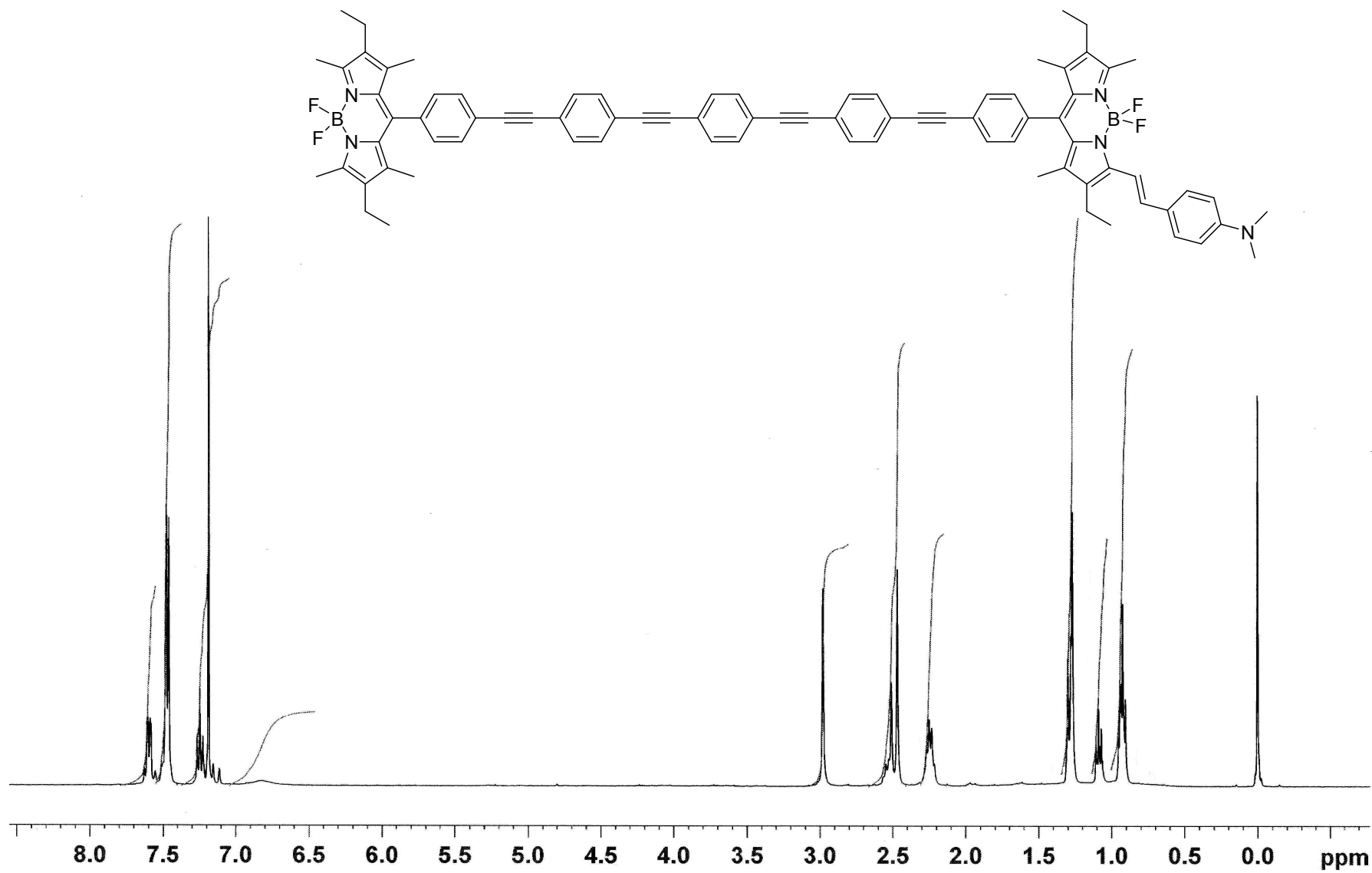
www.ccic.ohio-state.edu

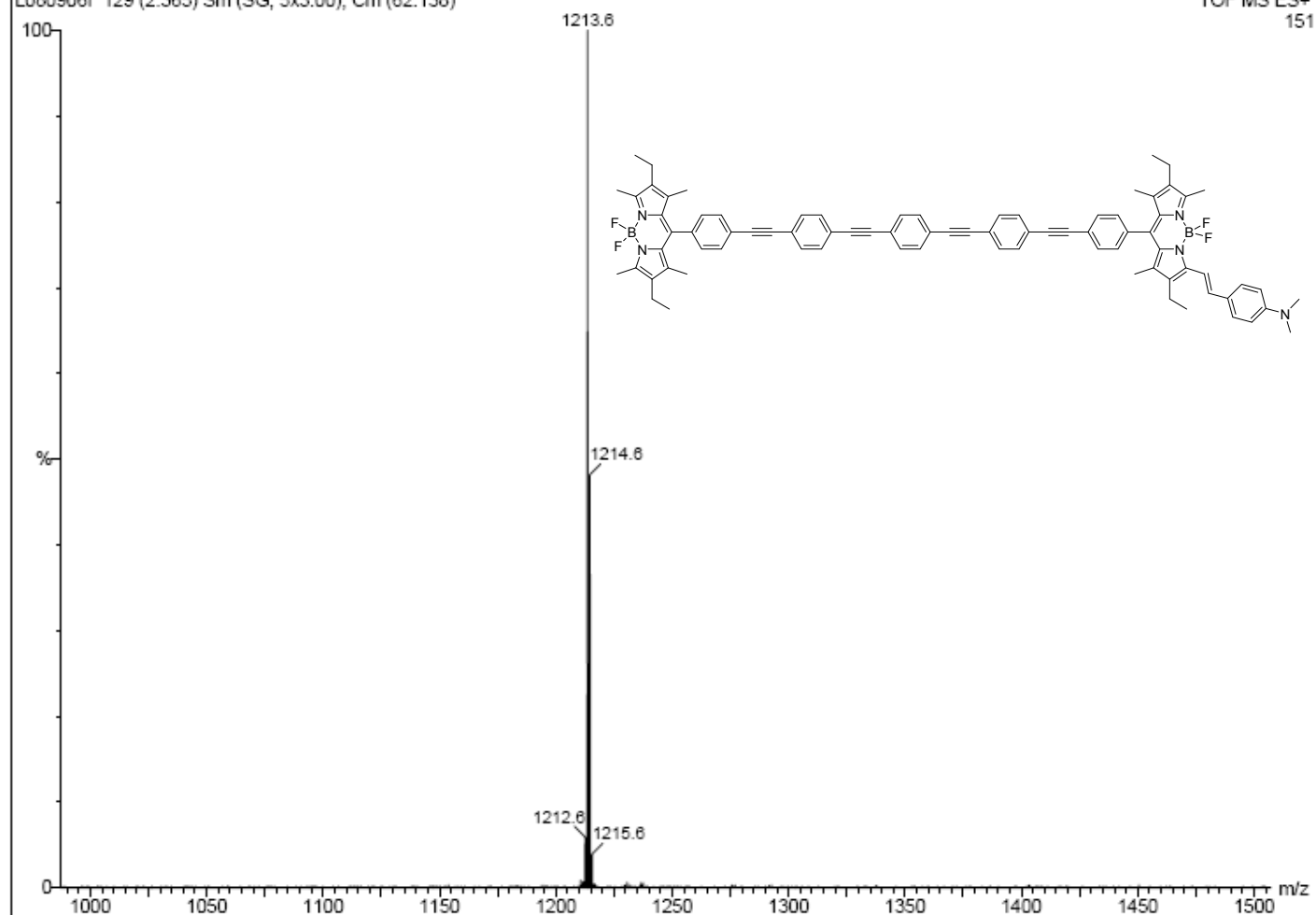
L080906G 215 (3.943) AM (Cen,4, 80.00, Ar,5000.0,0.00,1.00); Sm (SG, 5x3.00); Cm (206:224)

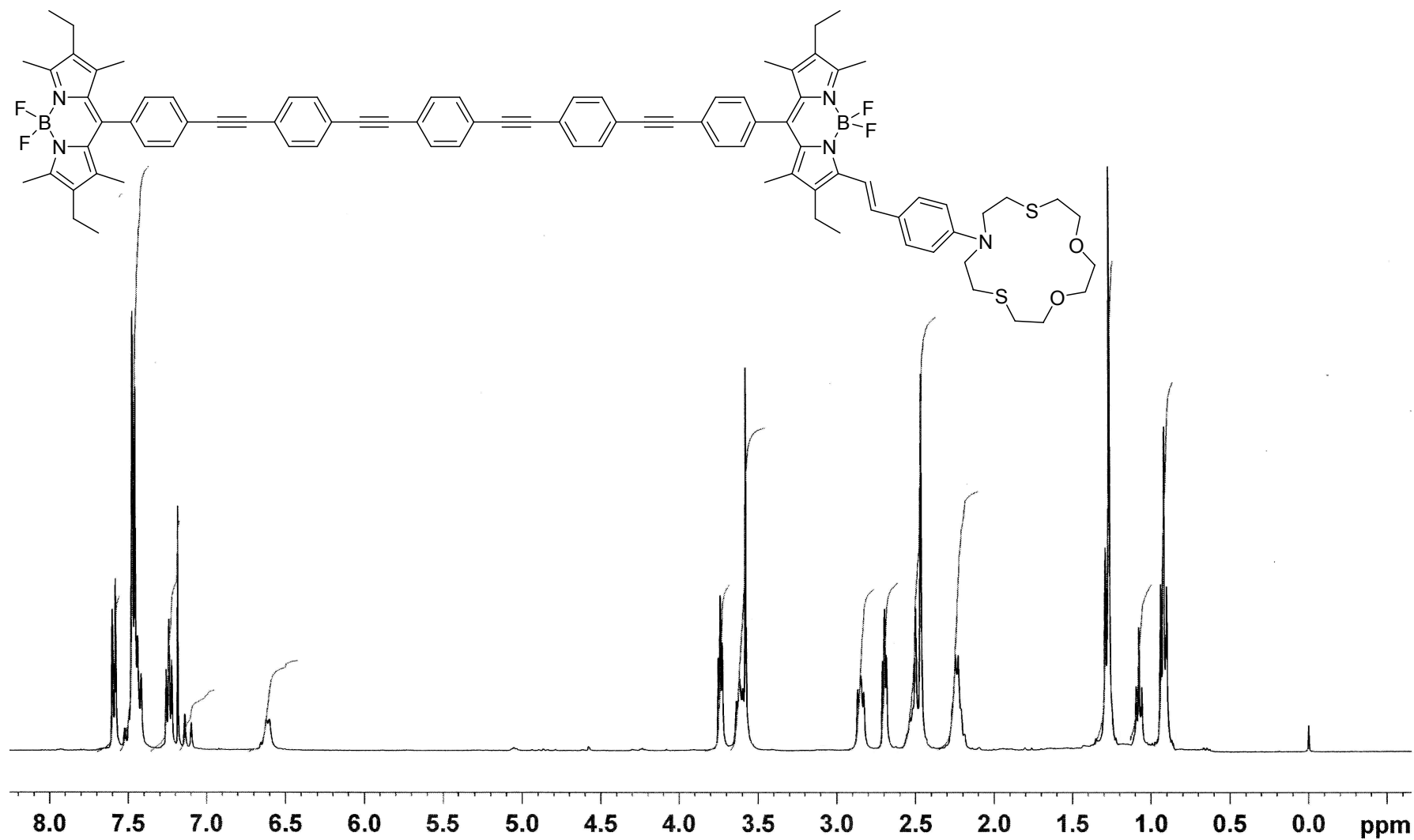
TOF MS ES+

353







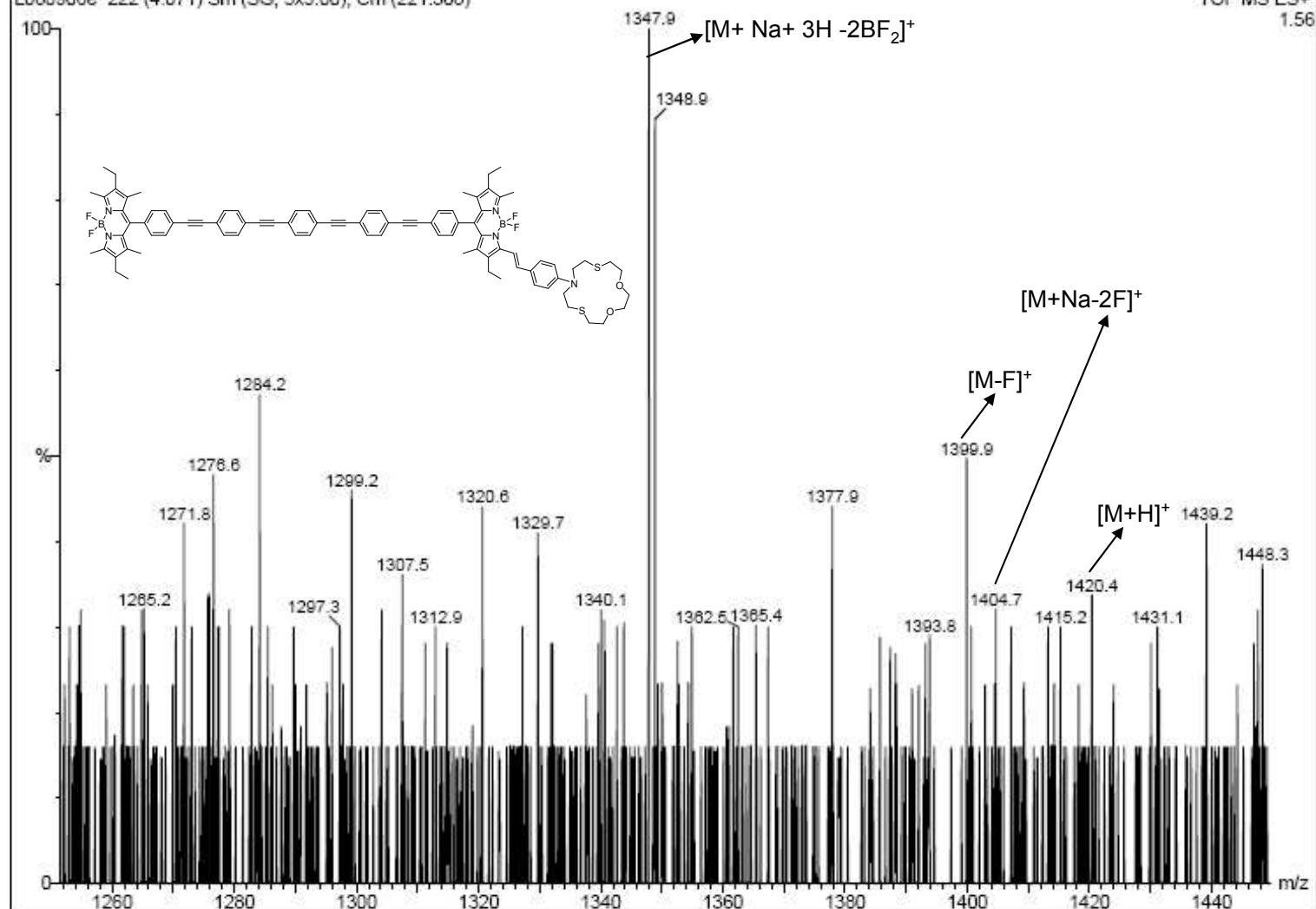




11117 AC-X5L Akkaya/METU
LCT electrospray
L080906e 222 (4.071) Sm (SG, 5x3.00); Cm (221:308)

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TOF MS ES+
1.56



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