Supporting Information

Bromo- and Glycosyl-Substituted BODIPYs for an Application in Photodynamic Therapy and Imaging

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S1 General remarks

All reactions were performed in standard round bottom flasks. DCM, HFIP, and methanol were purchased and used as received. Other solvents were purchased and distilled at reduced pressure. Purchased chemicals were used as received without further purification. All liquid reagents were added through syringes. Reactions were monitored by thin-layer chromatography (Merck, TLC Silica gel 60 F_{254}) and visualized under UV light (254 nm and 366 nm). Flash column chromatography was performed on silica gel (Fluka silica gel 60M, 40-63µm). NMR spectra were recorded with JEOL ECX400 and JEOL ECP500 Instruments. Multiplicity of the signals was assigned as follows: s = singlet, br s = broad singlet, d = doublet, t = triplet, dd = doublet of doublets, ddd = doublet of doublets of doublets, ddt = doublet of doublets of triplets, m = multiplet, m_c = centered multiplet. Chemical shifts are reported relative to $CDCl_3$ (^{1}H: δ = 7.26 ppm, ^{13}C: δ = 77.2 ppm) and THF-d_8 (^{1}H: δ = 3.58 ppm, ^{13}C: δ = 67.6 ppm). All ^{13}C NMR spectra are proton-decoupled and coupling constants are given in hertz (Hz). For a detailed peak assignment 2D spectra were measured (COSY, HMBC, and HMQC). HRMS analyses were carried out on an Agilent Technologies 6210 ESI-TOF (electrospray ionization, time of flight) instrument. IR spectra were measured with a JASCO FT/IR 4100 spectrometer equipped with a PIKE MIRacle™ ATR instrument. UV/Vis spectra were recorded on a SPECORD S300 UV/Vis spectrometer (Analytik Jena) in quartz cuvettes (1 cm length). The fluorescence spectra of the BODIPYs were recorded with a JASCO FP 6500 spectrofluorometer in quartz cuvettes (1 cm length). Specified melting points were recorded on a Reichert Thermovar Apparatus and are not corrected.

Core-unsubstituted BODIPYs **1** – **16**[1], 1,3,5,7-tetramethyl-substituted BODIPYs **17** – **29**[2,3], 2-bromo-substituted BODIPYs **30** – **37**[3], and 2,6-dibromo-substitituted BODIPYs **38** – **50**[3] were prepared according to the literature.

S2 Cellular testing

S2.1 Cellular assays

All cell lines used were purchased from DSMZ, except for the A-253 cell line which was purchased from ATCC. Human epidermoid carcinoma (A-431), human epithelial tongue squamous cell carcinoma (CAL-27), human colorectal adenocarcinoma (HT-29), and submaxillary salivary gland epidermoid carcinoma (A-253) were grown in Dulbecco's modified eagle medium (DMEM) with 10% heat inactivated FCS, 1% penicillin (10,000 IU) and

streptomycin (10,000 µg mL^{-1}). A stock solution (2 mM) of the BODIPY was prepared in DMSO and kept in the dark at 4 °C. DMEM (without phenol red) with 10% fetal calf serum (FCS) was used for further dilution to reach a concentration of 2 or 10 µM of the BODIPY, respectively. In microplates 2 × 10^4 cells per well were seeded in fresh medium (DMEM without phenol red containing 10% FCS) with 2 µM or 10 µM solution of the BODIPY and incubated for 24 h. After an exchange of medium (to remove any BODIPY not taken up by the cells), the irradiation was performed at rt with a white light source (KL 2500 LCD, Schott) at an energy dose of approximately 50 J cm^{-} the absorbance was m of 490 nm.

S2.2 Cellular tests of core

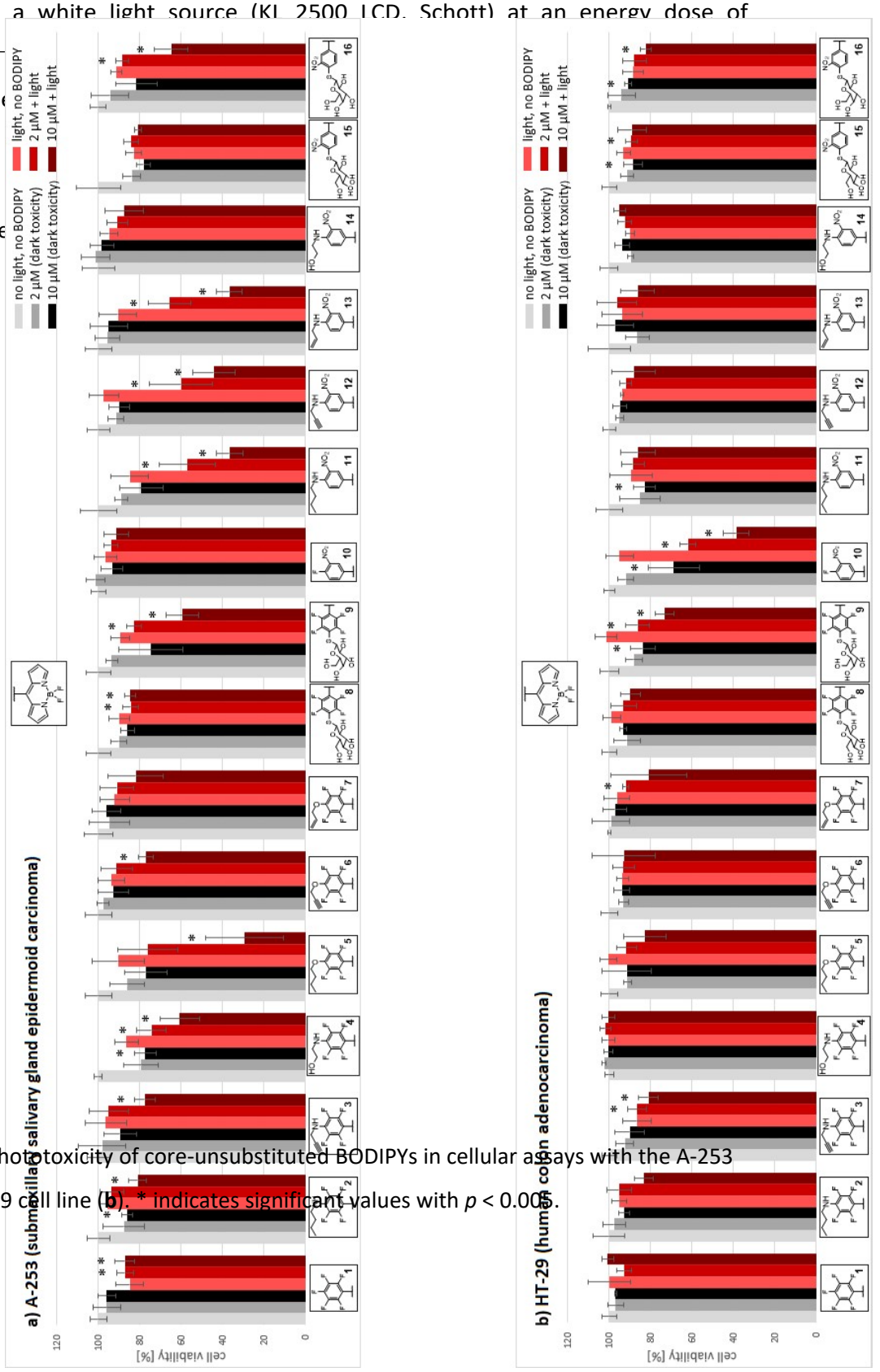


Figure S2.2: Dark- and phototoxicity of core-unsubstituted BODIPYs in cellular assays with the A-253 cell line (**a**) and the HT-29 cell line (**b**). * indicates significant values with $p < 0.005$.

S2.3 Cellular tests of 1,3,

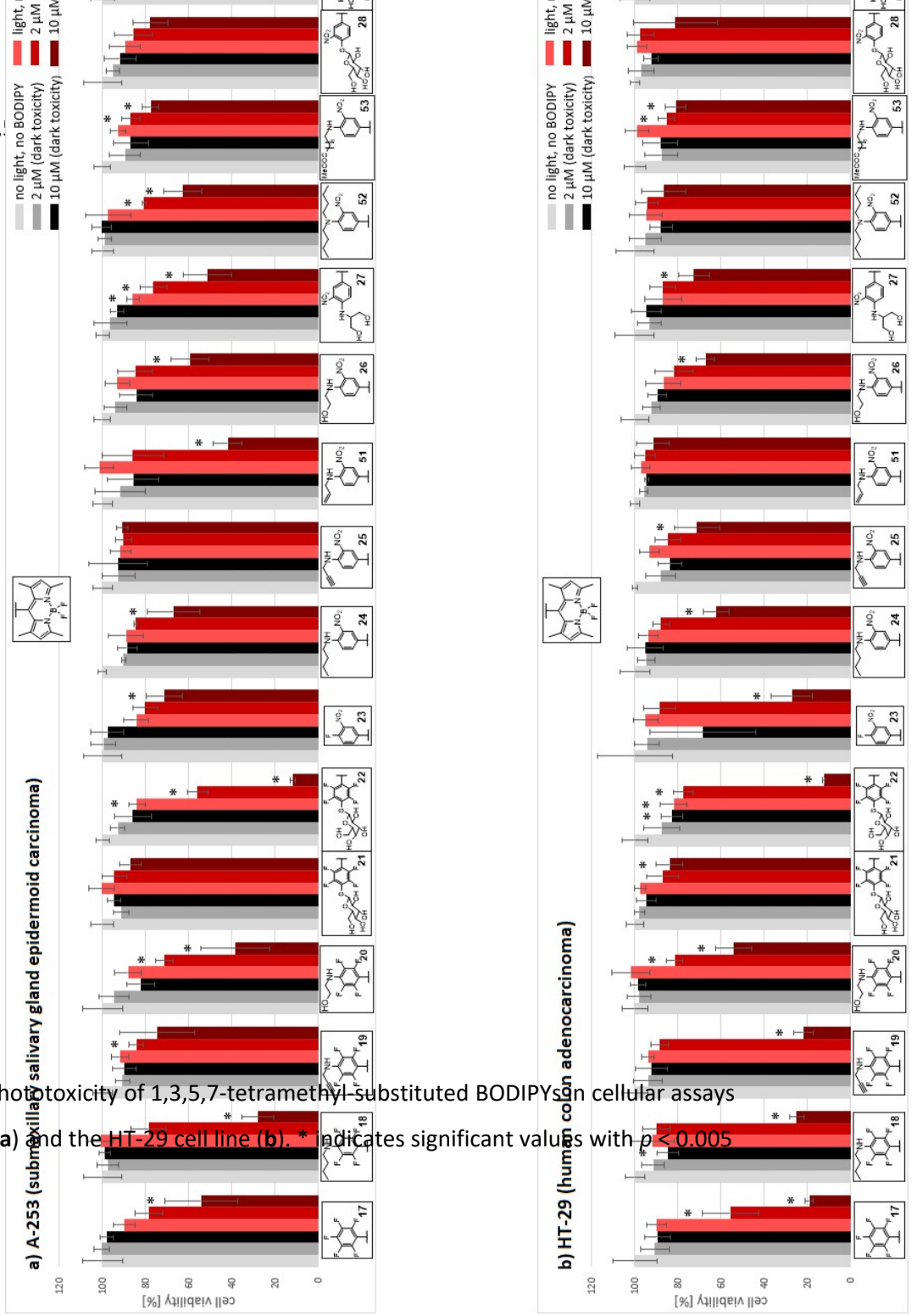


Figure S2.3: Dark- and phototoxicity of 1,3,5,7-tetramethyl-substituted BODIPYs in cellular assays with the A-253 cell line (**a**) and the HT-29 cell line (**b**). * indicates significant values with $p < 0.005$

S2.4 Cellular tests of 2,6-

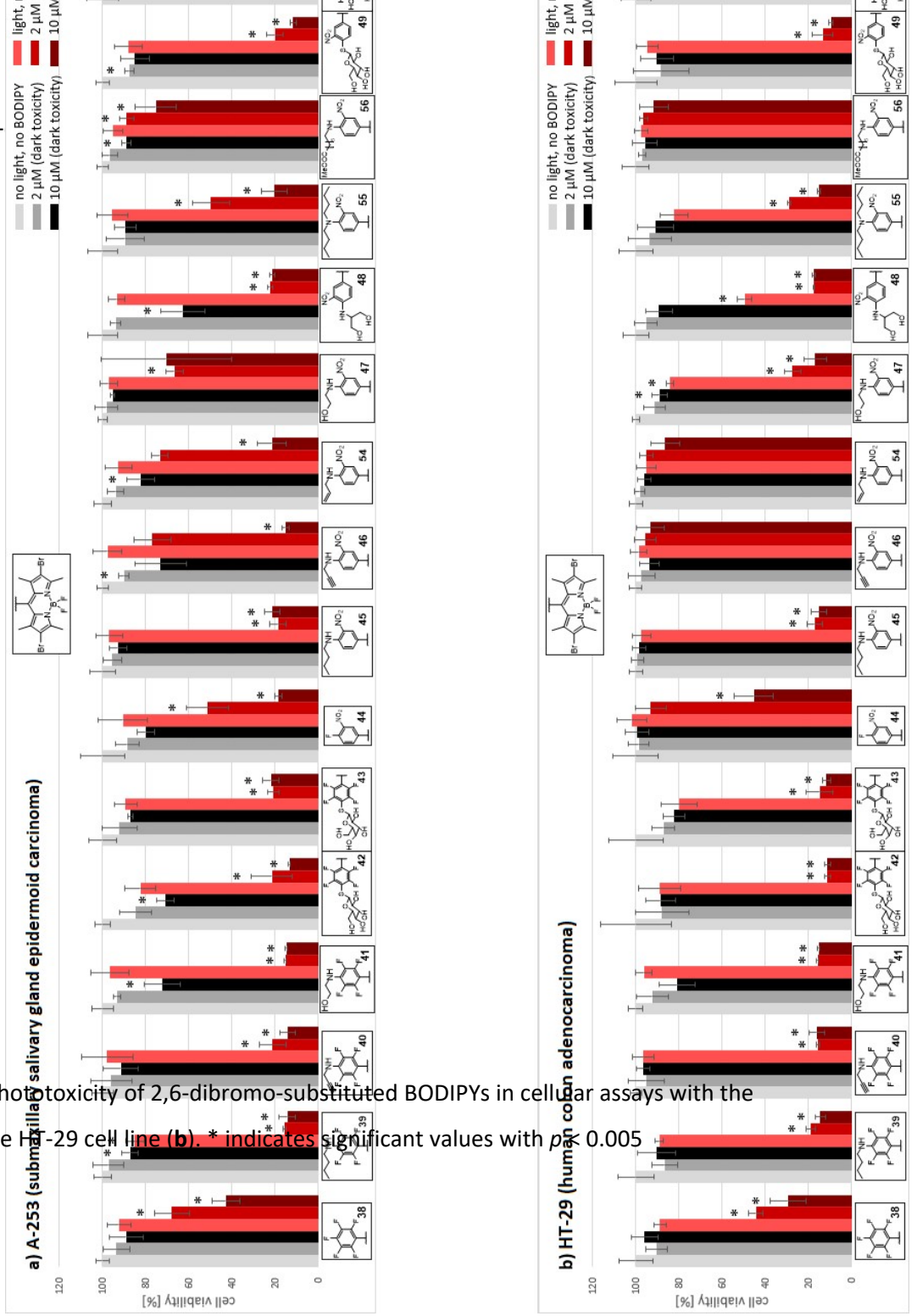


Figure S2.4: Dark- and phototoxicity of 2,6-dibromo-substituted BODIPYs in cellular assays with the A-253 cell line (**a**) and the HT-29 cell line (**b**). * indicates significant values with $p < 0.005$

S2.5 Cellular tests of 2-bromo-substituted BODIPYs against A-253 and HT-29

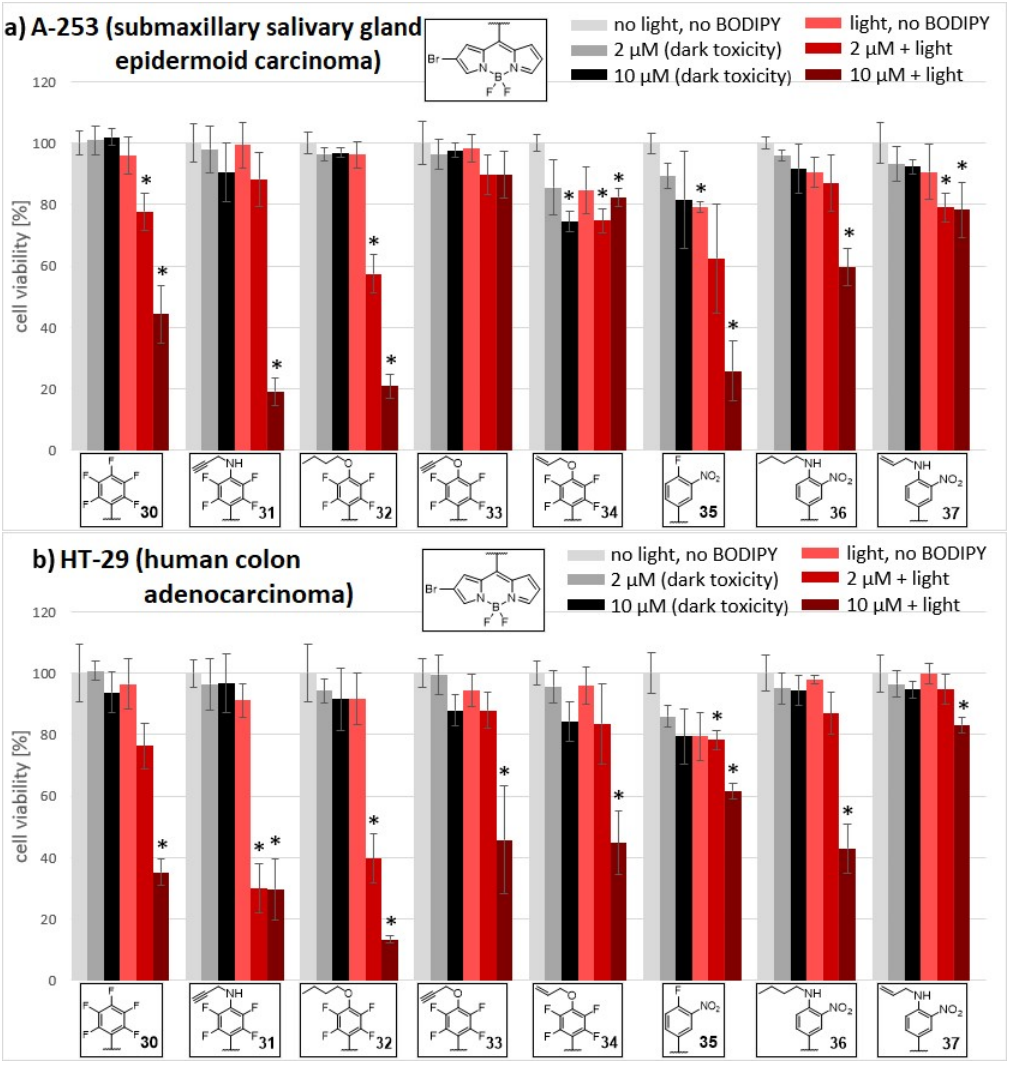
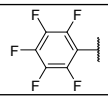
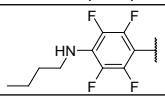
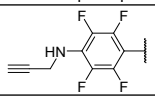
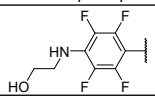
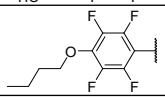
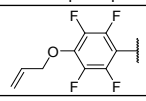
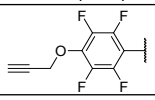
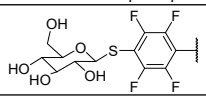
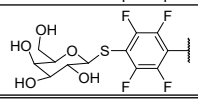
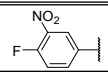
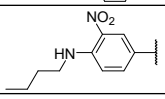
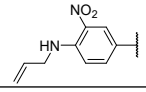
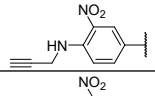
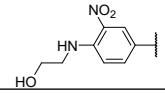
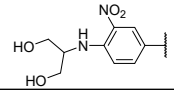
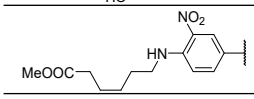
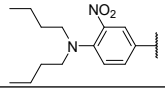
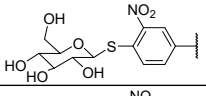
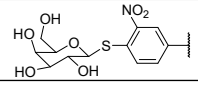


Figure S2.5: Dark- and phototoxicity of 2-bromo-substituted BODIPYs in cellular assays with the A-253 cell line (**a**) and the HT-29 cell line (**b**). * indicates significant values with $p < 0.005$.

S2.6 Overview on the phototoxic activity of the four series of BODIPYs regarding the different substitutions. *Highlighted compounds showed a cell viability reduction of > 40 % at 10 µM in at least two cell lines under irradiation (X indicates structural combinations that have not been synthesized).*

Aryl substituent at the 8-position	***BODIPY***	***1,3,5,7-Tetramethyl-BODIPY***	***2,6-Dibromo-1,3,5,7-tetramethyl-BODIPY***	***2-Bromo-BODIPY***
	**1**	**17**	**38**	**30**
	**2**	**18**	**39**	X
	**3**	**19**	**40**	**31**
	**4**	**20**	**41**	X
	**5**	X	X	**32**
	**6**	X	X	**33**
	**7**	X	X	**34**
	**8**	**21**	**42**	X
	**9**	**22**	**43**	X
	**10**	**23**	**44**	**35**
	**11**	**24**	**45**	**36**
	**12**	**51**	**54**	X
	**13**	**25**	**46**	**37**
	**14**	**26**	**47**	X
	**15**	X	**27**	**48**	X
	**16**	X	**52**	**55**	X
	**17**	X	**53**	**56**	X
	**18**	**28**	**49**	X
	**19**	**29**	**50**	X

S3 Confocal laser scanning microscopy (cLSM) for cellular uptake analysis

The uptake of the BODIPYs in A-431 cells was analyzed after 4 h and 24 h by confocal laser scanning microscopy (cLSM). A-431 cells were routinely cultivated as described above (S2.1). For the uptake study, 270 µl of a A-431 cell suspension in DMEM were seeded in each well of an 8-well ibidi µ-slide (50,000 cells mL^{-1}). After 1 day, 30 µl of selected BODIPYs were added at final test concentration of 10 µM and incubated for 4 h and 24 h. After 24 h cell nuclei were stained with 1 µg mL^{-1} Hoechst 33342 (Life Technologies GmbH, Darmstadt, Germany). Then confocal images were taken by using an inverted confocal laser scanning microscope Leica DMI6000CSB SP8 (Leica, Wetzlar, Germany) with a 63x/1.4 HC PL APO CS2 oil immersion objective and the LAS X software.

S4 Nucleophilic substitution of BODIPY 31 with amines (51 – 53)

S4.1 General synthetic procedure

BODIPY **23** (1 equiv.) was dissolved in DCM and the corresponding amine (10 – 20 equiv.) was added. The mixture was stirred for 24 h. Afterwards, the mixture was diluted with EtOAc and washed with water several times. The organic layer was dried with Na_2SO_4, filtered, and evaporated to dryness. The crude product was purified by column chromatography; the main fraction was collected and evaporated to dryness.

S4.2 8-[3-Nitro-4-(*N*-2-prop-2-enylamino)phenyl]-1,3,5,7-tetramethyl-4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene (**51**):

BODIPY **51** was prepared according to the general synthetic procedure. BODIPY **23** (132 mg, 0.34 mmol) and allylamine (0.3 mL, 3.40 mmol, 10 equiv.) were dissolved in 10 mL of DCM. After column purification (chromatography, silica gel, *n*-hexane/EtOAc = 9/1, v/v); BODIPY **51** was obtained as an orange solid (124 mg, 0.29 mmol, 86%).

^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) = 1.53 (s, 6H, Me), 2.54 (s, 6H, Me), 4.05 (t, J = 5.0 Hz, 2H, CH_2), 5.29–5.36 (m, 2H, **H_2C**=CH-), 5.95–6.02 (m, 3H, $H_{Pyrrole}$ + H_2C=**CH**-), 6.98 (d, J = 8.8 Hz, 1H, Ar-H_{meta}), 7.30 (dd, J = 8.8, 2.2 Hz, 1H, Ar-H_{ortho}), 8.14 (d, J = 2.1 Hz, 1H, Ar-H_{ortho}), 8.28 (t, J = 5.8 Hz, 1H, NH).

^{13}C NMR (126 MHz, $CDCl_3$): δ (ppm) = 14.7 (Me), 15.3 (Me), 45.6 (CH_2), 115.2 (Ar-C_{meta}), 117.7 (**H_2C**=CH), 121.6 ($CH_{Pyrrole}$), 121.8*, 126.8 (Ar-C_{ortho}), 131.9 ($C_{Pyrrole}$), 132.1 (C_{meso}), 132.7 (H_2C=**CH**-), 136.0 (Ar-C_{ortho}),

139.2*, 142.7 ($C_{Pyrrole}$), 145.3 (Ar-C_{para}), 156.1 ($C_{Pyrrole}$). *These signals could not be assigned exactly to corresponding carbon atoms. They belong to the Ar-C_{ipso} and the Ar-C_{nitro} of the aryl moiety.

^{19}F NMR (376 MHz, $CDCl_3$): δ (ppm) = -145.57 – -146.59 (m_c, 2F, BF_2).

HRMS (ESI-TOF, MeOH): *m/z* calcd. for $C_{22}H_{23}BF_2N_4O_2Na^+$ $[M+Na]^+$: 447.1774, found: 447.1796 , *m/z* calcd. for $C_{22}H_{23}BF_2N_4O_2K^+$ $[M+K]^+$: 463.1514, found: 463.1543, *m/z* calcd. for $C_{44}H_{46}B_2F_4N_8O_4Na^+$ $[2M+Na]^+$: 871.3656, found: 871.3699, *m/z* calcd. for $C_{44}H_{46}B_2F_4N_8O_4K^+$ $[2M+K]^+$: 887.3396, found: 887.3442.

IR (ATR): $\tilde{\nu}$ (cm^{-1}) = 3590 [ν(NH)], 2920 [$\nu(CH_2)$], 1628 [ν(C=N), ν(C=C)], 1543 [$\nu_{as}(NO_2)$], 1527 [δ(Ar-C)], 1466 [$\delta(CH_2)$, δ(Me)], 1349 [$\nu_{sym}(NO_2)$], 1061 [ν(BF)], 758 [δ(HC=CH)].

UV/vis (DCM): λ_{max} (nm) [log (ε/L mol^{-1} cm^{-1})] = 504 [4.83].

Fluorescence (DCM): λ_{max} (nm) = 517 at $\lambda_{Excitation}$ (nm) 490.

M.P. (°C): 176-179.

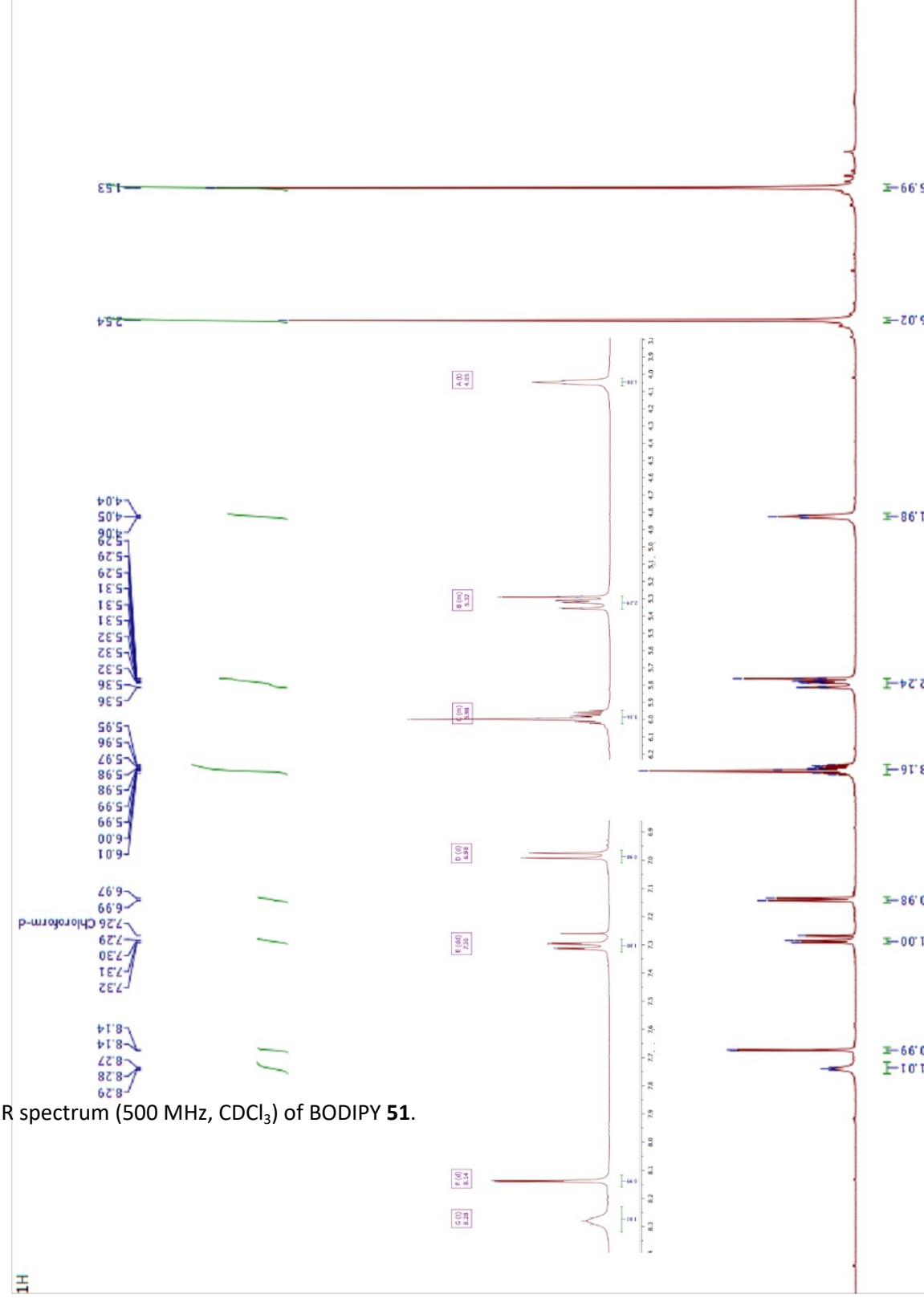
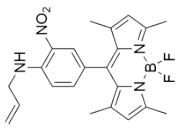


Figure S4.2.1: ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of BODIPY **51**.



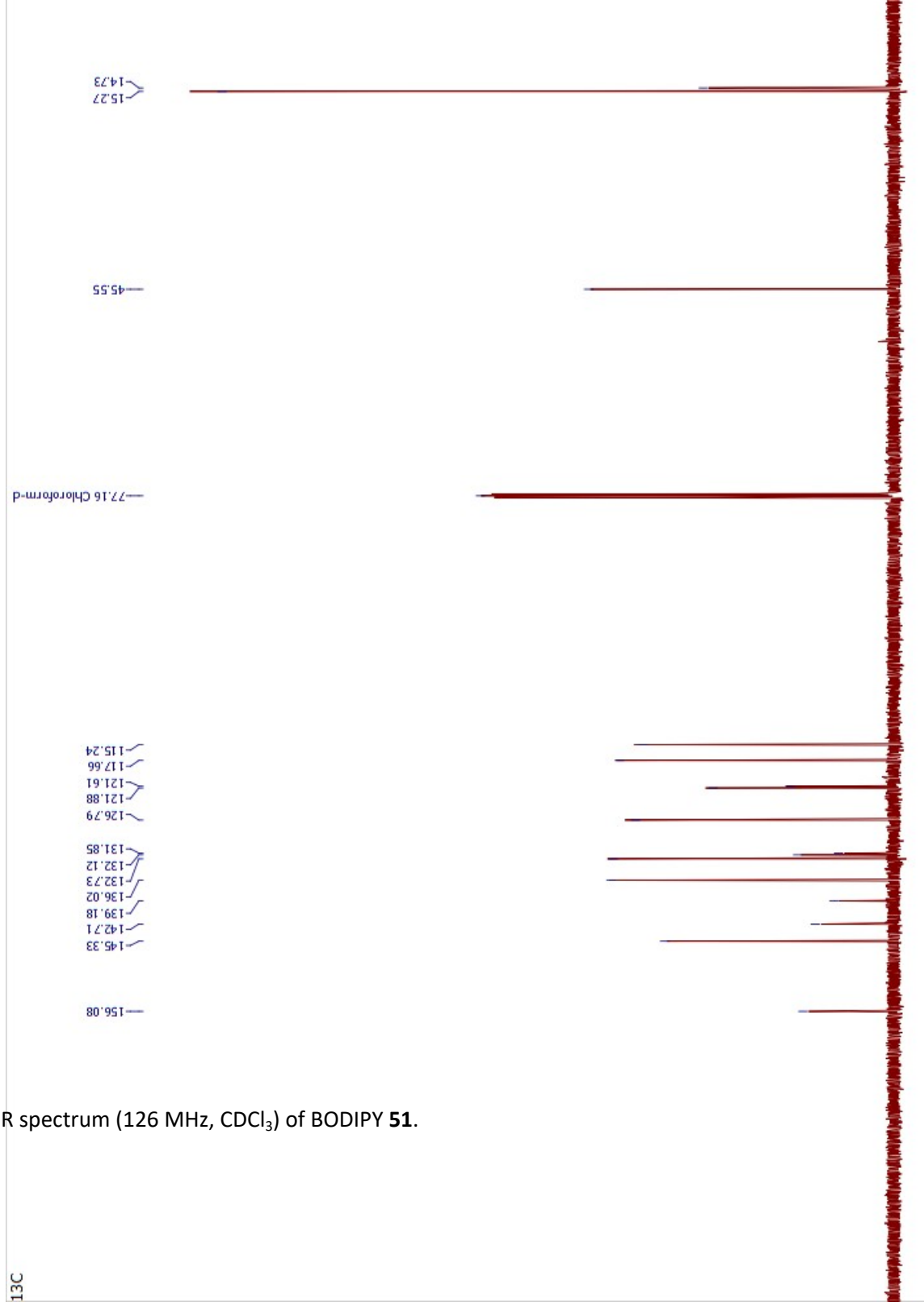
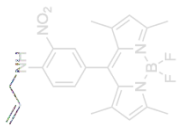


Figure S4.2.2: ^{13}C NMR spectrum (126 MHz, $CDCl_3$) of BODIPY **51**.



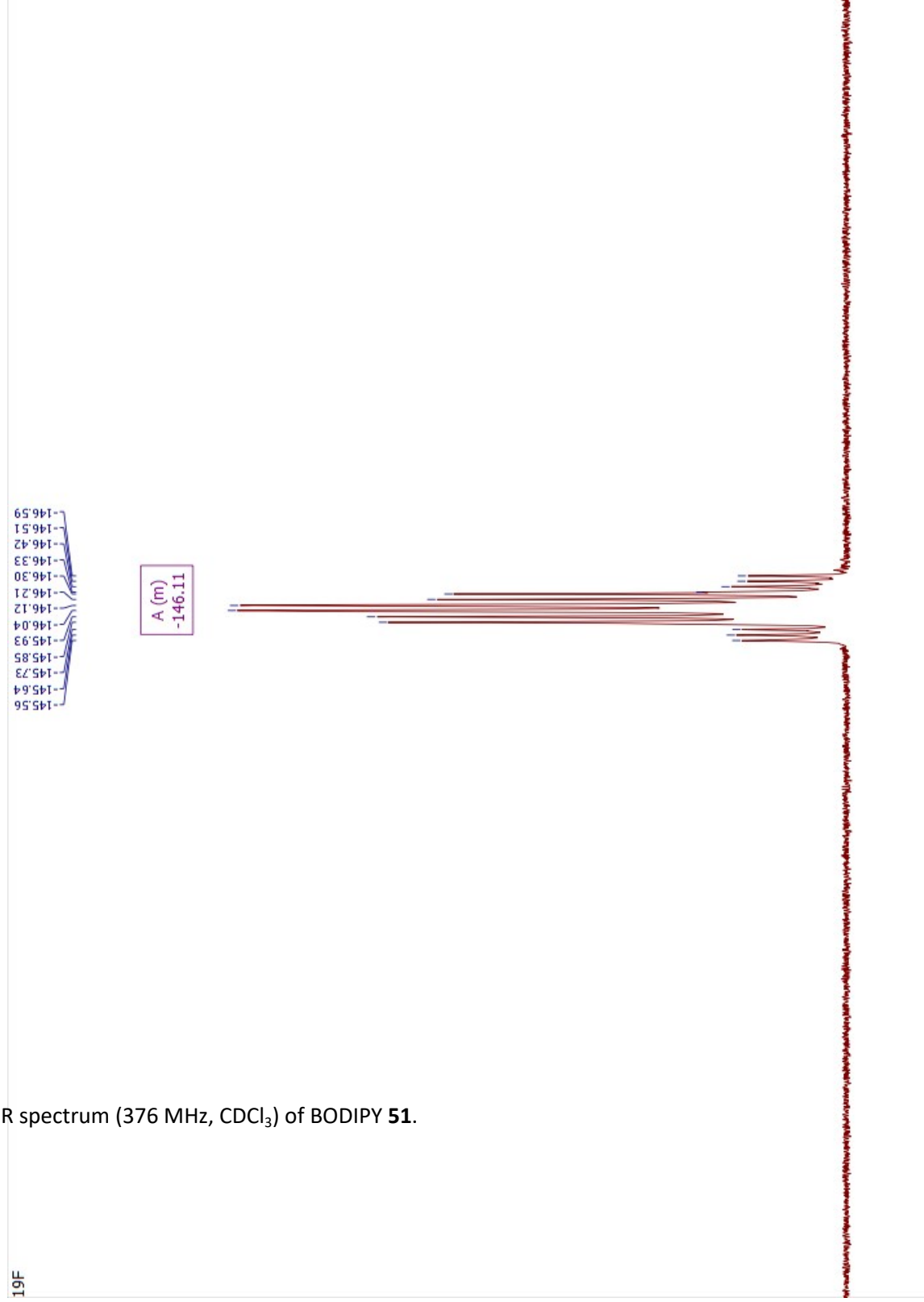
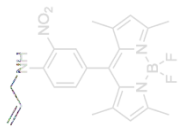


Figure S4.2.3: ^{19}F NMR spectrum (376 MHz, $CDCl_3$) of BODIPY **51**.



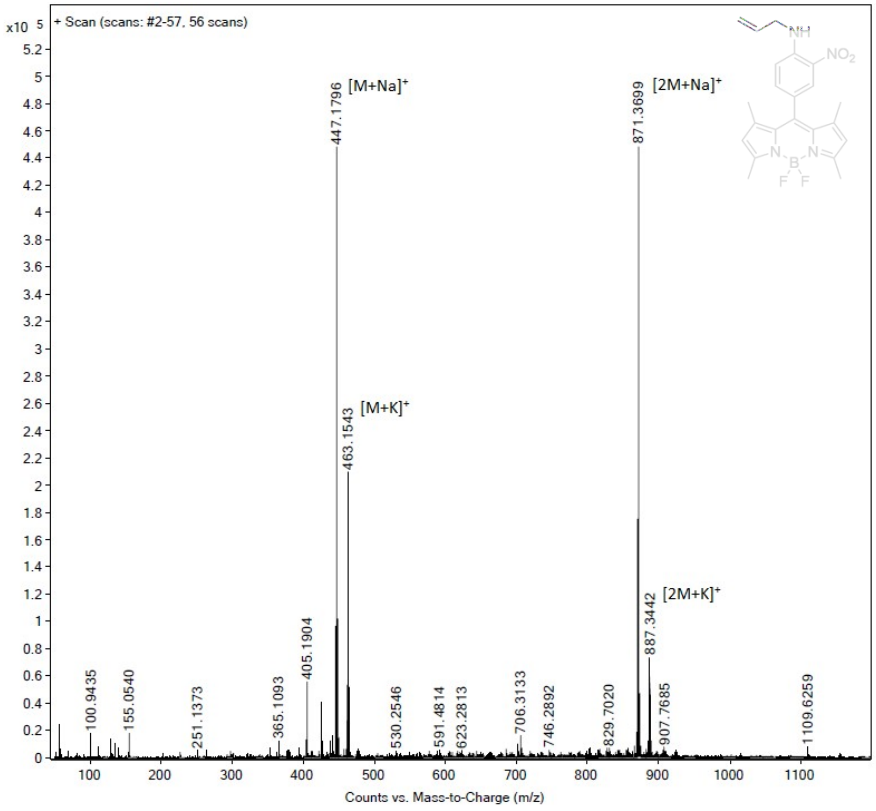


Figure S4.2.4: HRMS spectrum (ESI+) of BODIPY **51**.

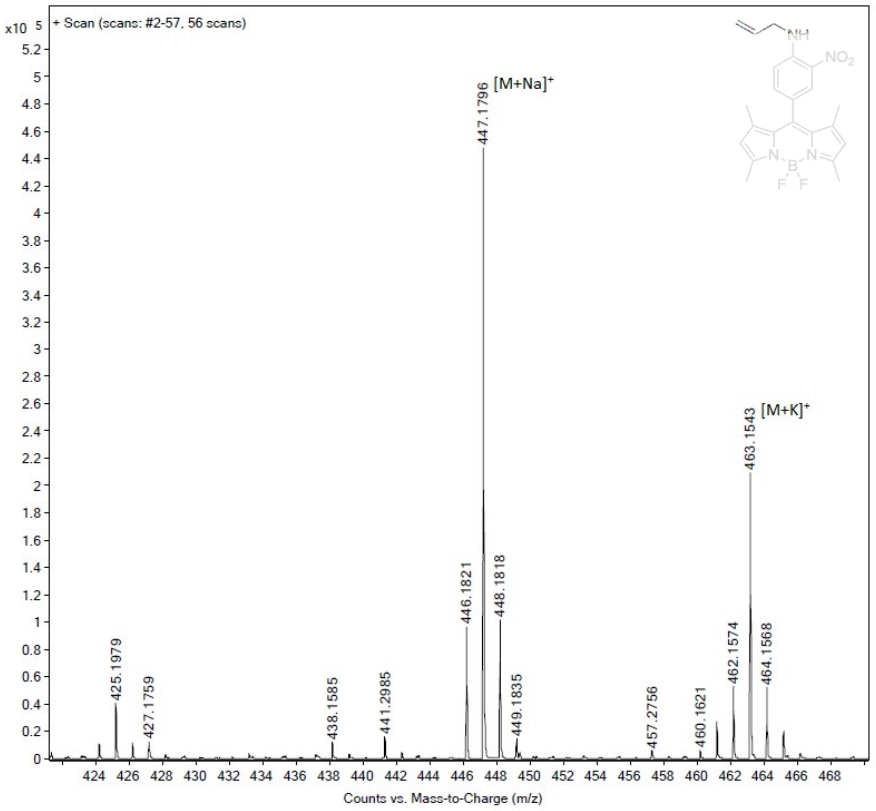


Figure S4.2.5: HRMS spectrum (ESI+) of BODIPY **51**.

S4.3 8-[4-(*N*-6-Methoxy-6-oxohexylamino)-3-nitrophenyl]-1,3,5,7-tetramethyl-4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene (**52**):

BODIPY **52** was prepared according to the general synthetic procedure. BODIPY **23** (200 mg, 0.52 mmol), *N*,*N*-diisopropylethylamine (1.8 mL, 10.33 mmol, 20 equiv.), and methyl-6-aminohexanoate hydrochloride (939 mg, 5.17 mmol, 10 equiv.) were dissolved in 20 mL of DCM. After purification (column chromatography, silica gel, *n*-hexane/EtOAc = 9/1, v/v, then *n*-hexane/EtOAc = 4/1, v/v); BODIPY **52** was obtained as an orange solid (80 mg, 0.16 mmol, 30%).

^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) = 1.49–1.57 (m, 8H, Me + CH_2), 1.70–1.76 (m, 2H, CH_2), 1.77–1.83 (m, 2H, CH_2), 2.36 (t, J = 7.4 Hz, 2H, CH_2), 2.54 (s, 6H, Me), 3.36 (td, J = 7.1, 5.0 Hz, 2H, CH_2), 3.67 (s, 3H, Me_{COOMe}), 5.99 (s, 2H, $H_{Pyrrole}$), 6.98 (d, J = 8.8 Hz, 1H, Ar-H_{meta}), 7.31 (dd, J = 8.7, 2.2 Hz, 1H, Ar-H_{ortho}), 8.11–8.14 (m, 2H, Ar-H_{ortho} + NH).

^{13}C NMR (126 MHz, $CDCl_3$): δ (ppm) = 14.7 (Me), 15.3 (Me), 24.6 (CH_2), 26.7 (CH_2), 28.7 (CH_2), 33.9 (CH_2), 43.1 (CH_2), 51.7 (Me_{COOMe}), 114.8 (Ar-C_{meta}), 121.5*, 121.6 ($CH_{Pyrrole}$), 126.9 (Ar-C_{ortho}), 131.9 ($C_{Pyrrole}$), 131.9 (C_{meso}), 136.1 (Ar-C_{ortho}), 139.3*, 142.7 ($C_{Pyrrole}$), 145.5 (Ar-C_{para}), 156.1 ($C_{Pyrrole}$), 173.95 (CO). *These signals could not be assigned exactly to corresponding carbon atoms. They belong to the Ar-C_{ipso} and the Ar-C_{nitro} of the aryl moiety.

^{19}F NMR (376 MHz, $CDCl_3$): δ (ppm) = -145.57 – -146.63 (m_c, 2F, BF_2).

HRMS (ESI-TOF, MeOH): *m/z* calcd. for $C_{26}H_{31}BFN_4O_4^+$ $[M\text{-}F]^+$: 493.2417, found: 493.2410, *m/z* calcd. for $C_{26}H_{31}BF_2N_4O_4Na^+$ $[M+Na]^+$: 535.2299, found: 535.2298.

IR (ATR): $\tilde{\nu}$ (cm^{-1}) = 3377 [ν(NH)], 2929 [ν(CH_2), ν(Me)], 2858 [ν(CH)], 1734 [ν(COOMe)], 1626 [ν(C=N), ν(C=C)], 1542 [ν_{as}(NO_2)], 1526 [δ(Ar-C)], 1468 [δ(CH_2), δ(Me)], 1304 [ν_{sym}(NO_2)], 1075 [ν(BF)], 758 [δ(HC=CH)]. **UV/vis (DCM):** λ_{max} (nm) [log (ε/L mol^{-1} cm^{-1})] = 504 [4.66].

Fluorescence (DCM): λ_{max} (nm) = 518 at $\lambda_{Excitation}$ (nm) 490.

M.P. (°C): 123-125.

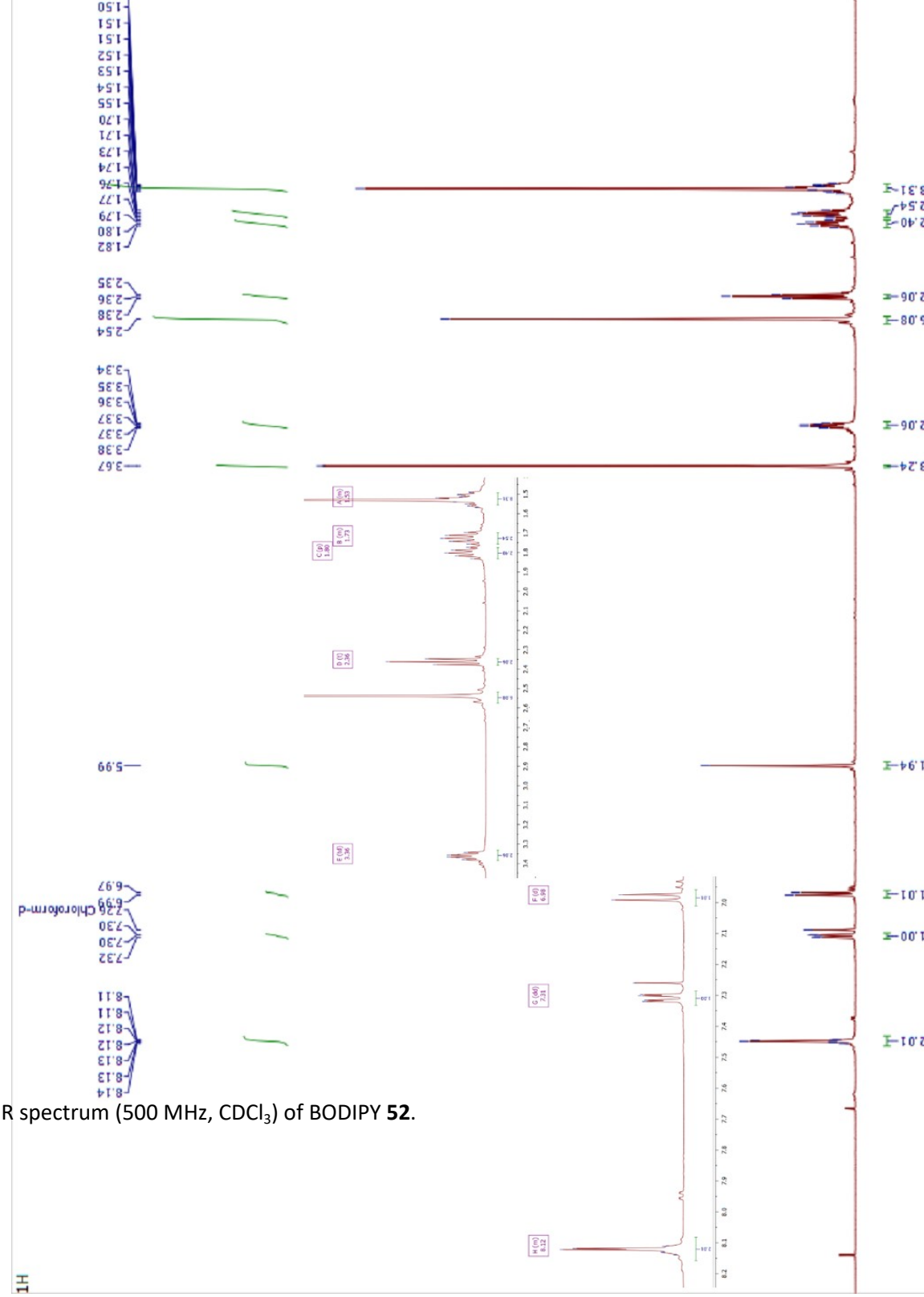
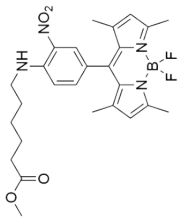


Figure S4.3.1: ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of BODIPY **52**.



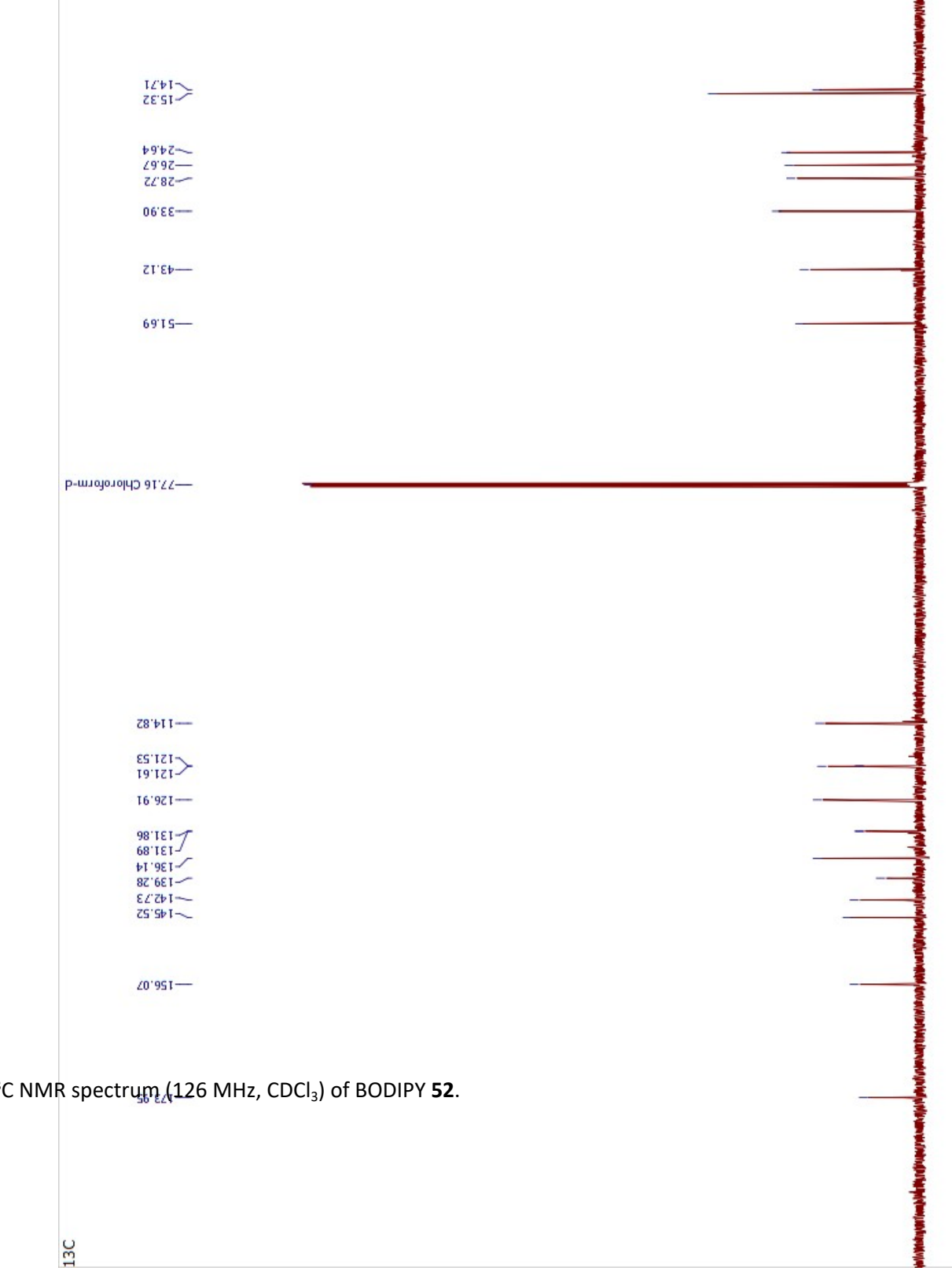


Figure S4.3.2: ^{13}C NMR spectrum (126 MHz, $CDCl_3$) of BODIPY **52**.

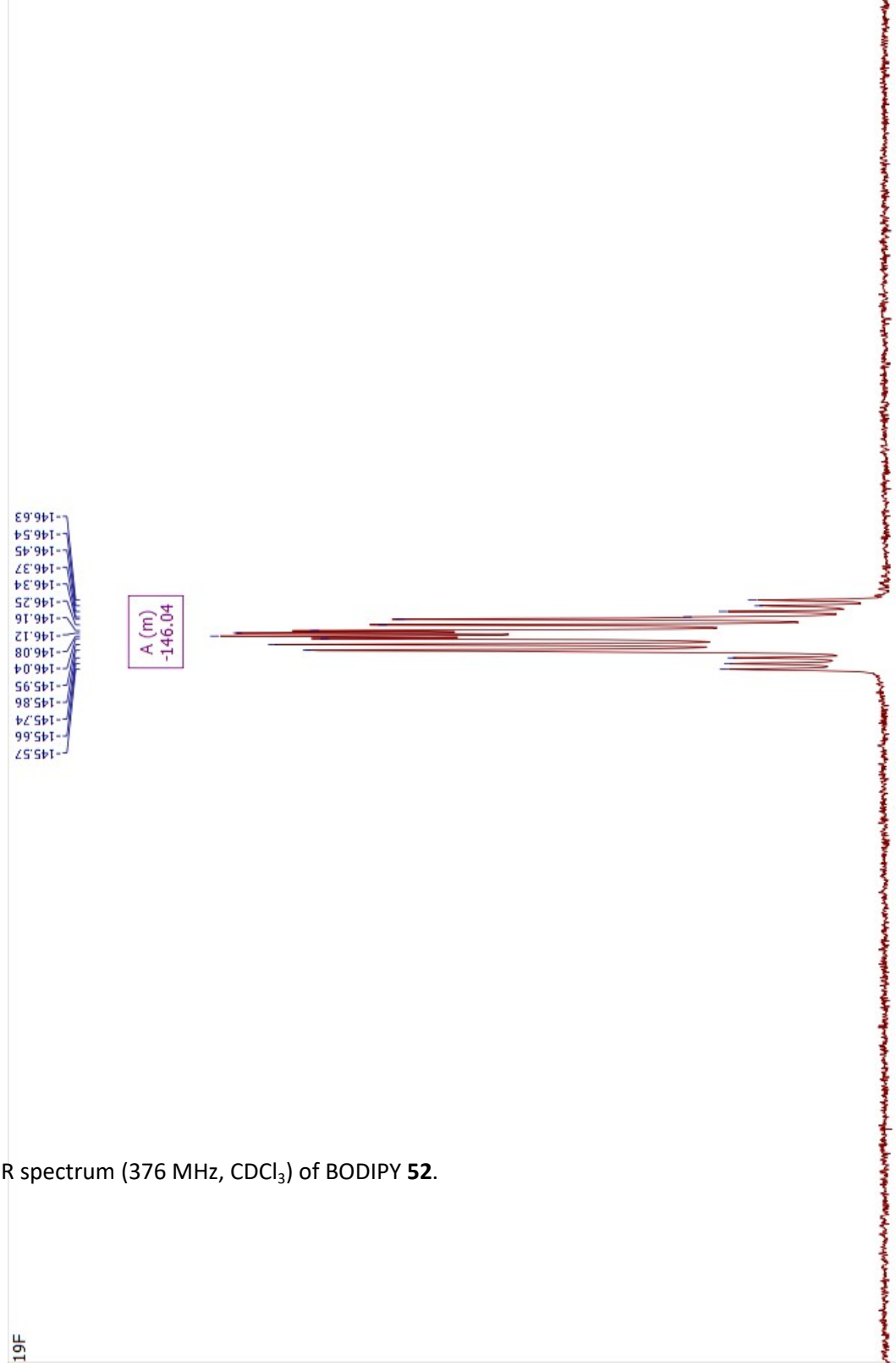


Figure S4.3.3: ^{19}F NMR spectrum (376 MHz, $CDCl_3$) of BODIPY **52**.

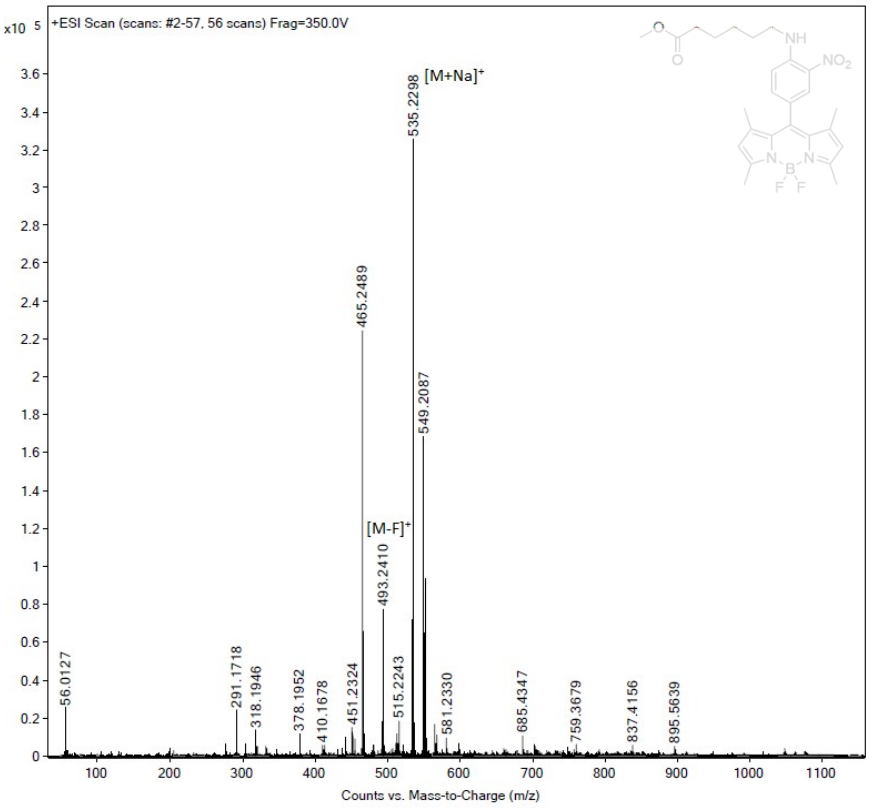


Figure S4.3.4: HRMS spectrum (ESI+) of BODIPY **52**.

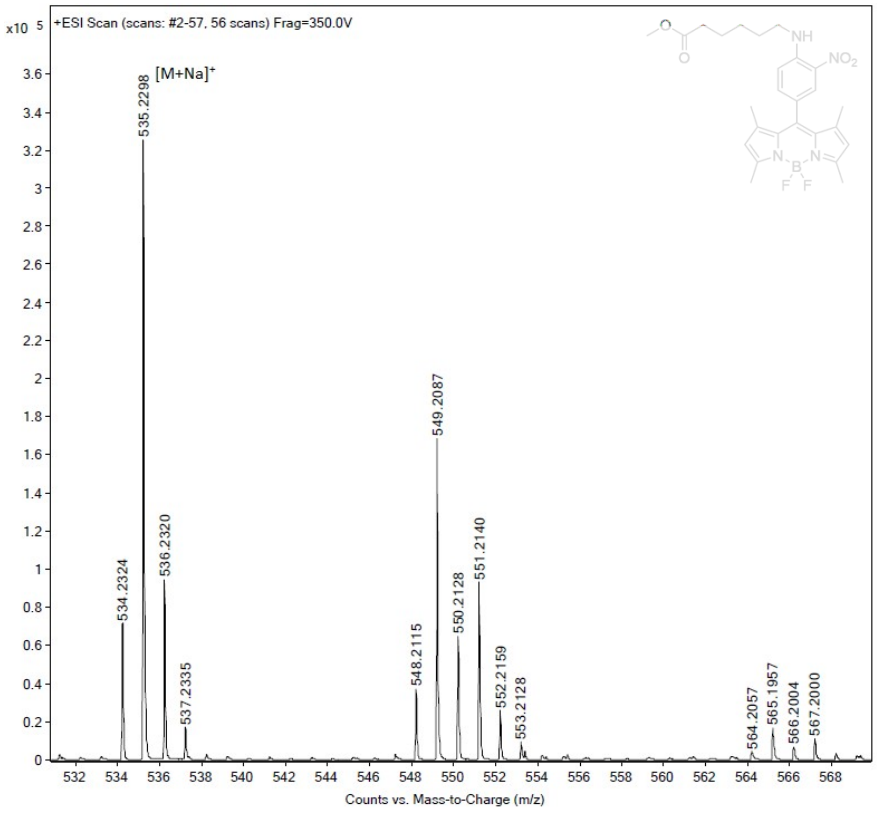


Figure S4.3.5: HRMS spectrum (ESI+) of BODIPY **52**.

S4.4 8-[4-(*N*,*N*-Dibutylamino)-3-nitrophenyl]-1,3,5,7-tetramethyl-4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene (**53**):

BODIPY **53** was prepared according to the general synthetic procedure. BODIPY **23** (200 mg, 0.52 mmol) and *N*,*N*-dibutylamine (1.60 mL, 9.66 mmol, 15 equiv.) were dissolved in 10 mL of DCM. After column purification (chromatography, silica gel, *n*-hexane/EtOAc = 9/1, v/v); BODIPY **53** was obtained as an orange solid (90 mg, 0.18 mmol, 35%).

^{1}H NMR (500 MHz, $CDCl_3$): δ (ppm) = 0.87 (t, J = 7.4 Hz, 6H, Me_{Butyl}), 1.25–1.32 (m, 4H, CH_2), 1.50–1.56 (m, 10H, CH_2 + Me) , 2.55 (s, 6H, Me), 3.20 (t, J = 7.3 Hz, 1H, CH_2), 6.00 (s, 2H, $H_{Pyrrole}$), 7.28 (br s, 2H, Ar-H_{meta} + Ar-H_{ortho}), 7.65 (t, J = 1.2 Hz, 1H, Ar-H_{ortho}).

^{13}C NMR (126 MHz, $CDCl_3$): δ (ppm) = 13.9 (Me_{Butyl}), 14.8 (Me), 14.99 (Me), 20.2 (CH_2), 29.5 (CH_2), 52.2 (CH_2), 121.7 ($CH_{Pyrrole}$), 122.6 (Ar-C_{meta}), 126.3 (Ar-C_{ortho}), 131.7 ($C_{Pyrrole}$), 132.7 (Ar-C_{ortho}), 138.96*, 142.1*, 142.8 ($C_{Pyrrole}$), 144.9 (Ar-C_{para}), 156.2 ($C_{Pyrrole}$). *These signals could not be assigned exactly to corresponding carbon atoms. They belong to the Ar-C_{ipso} and the Ar-C_{nitro} of the aryl moiety.

^{19}F NMR (376 MHz, $CDCl_3$): δ (ppm) = -145.61 – -146.64 (m_c, 2F, BF_2).

HRMS (ESI-TOF, MeOH): *m/z* calcd. for $C_{27}H_{35}BF_2N_4O_2Na^+$ $[M+Na]^+$: 519.2713, found: 519.2713.

IR (ATR): $\tilde{\nu}$ (cm^{-1}) = 2957 and 2929 [ν(CH_2), ν(Me)], 2871 [ν(CH)], 1613 [ν(C=N), ν(C=C)], 1545 [ν_{as}(NO_2)], 1512 [δ(Ar-C)], 1467 [δ(CH_2), δ(Me)], 1348 [ν_{sym}(NO_2)], 1082 [ν(BF)], 760 [δ(HC=CH)].

UV/vis (DCM): λ_{max} (nm) [log (ε/L mol^{-1} cm^{-1})] = 505 [4.62].

Fluorescence (DCM): λ_{max} (nm) = 515 at $\lambda_{Excitation}$ (nm) 490.

M.P. (°C): 95-99.

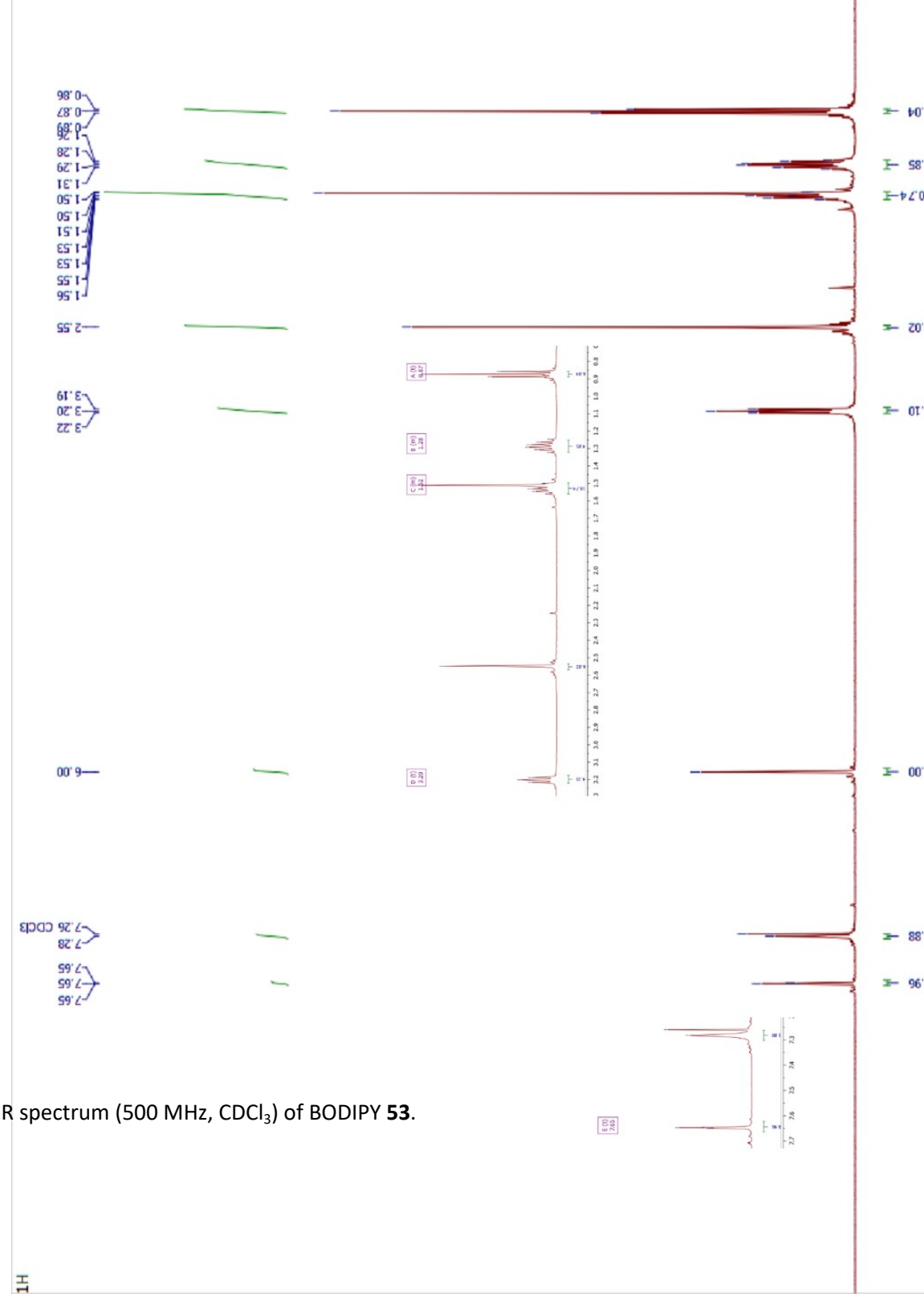
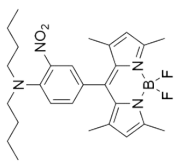


Figure S4.4.1: ^{1}H NMR spectrum (500 MHz, $CDCl_3$) of BODIPY **53**.



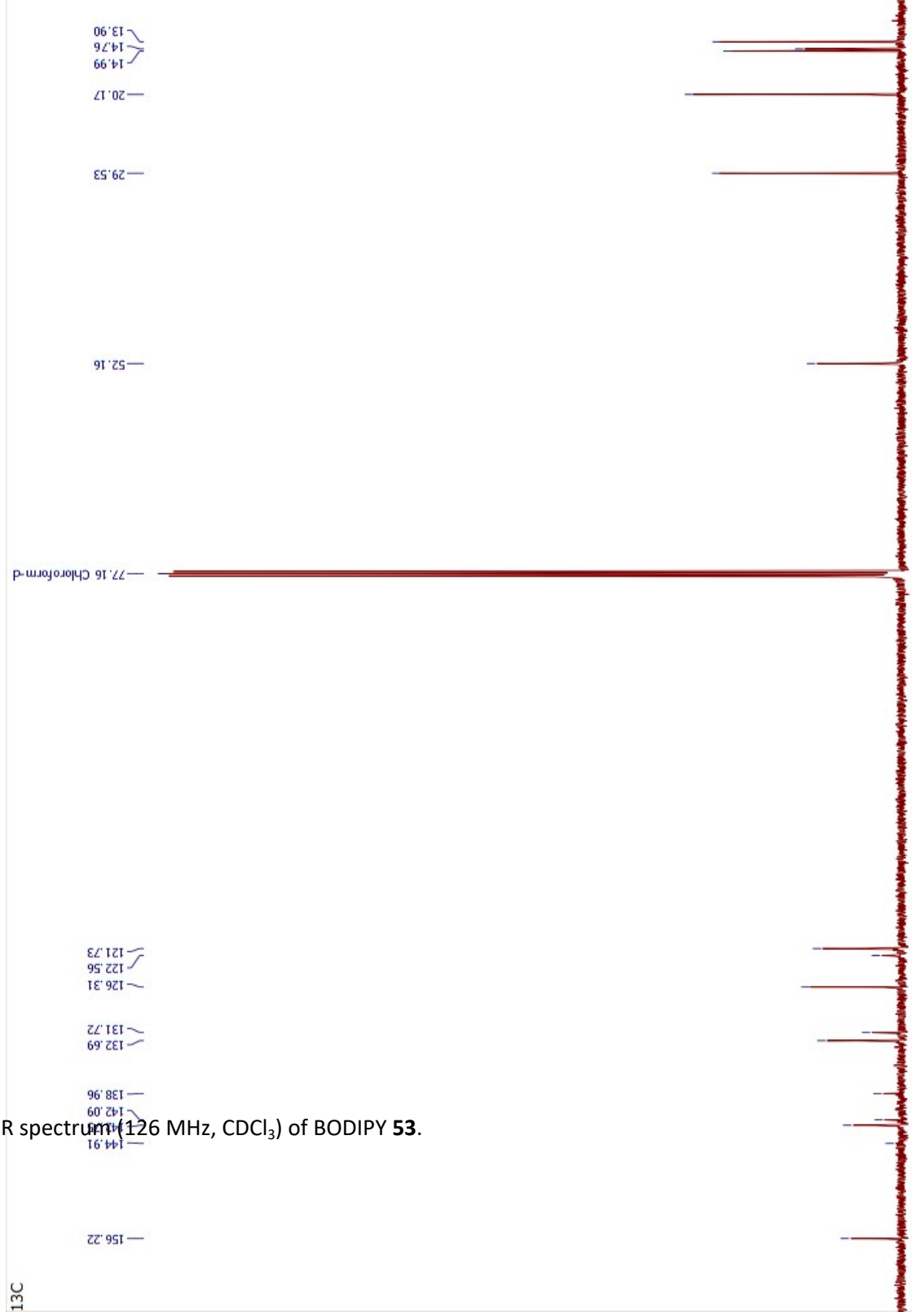
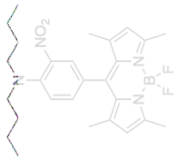


Figure S4.4.2: ^{13}C NMR spectrum (126 MHz, $CDCl_3$) of BODIPY **53**.



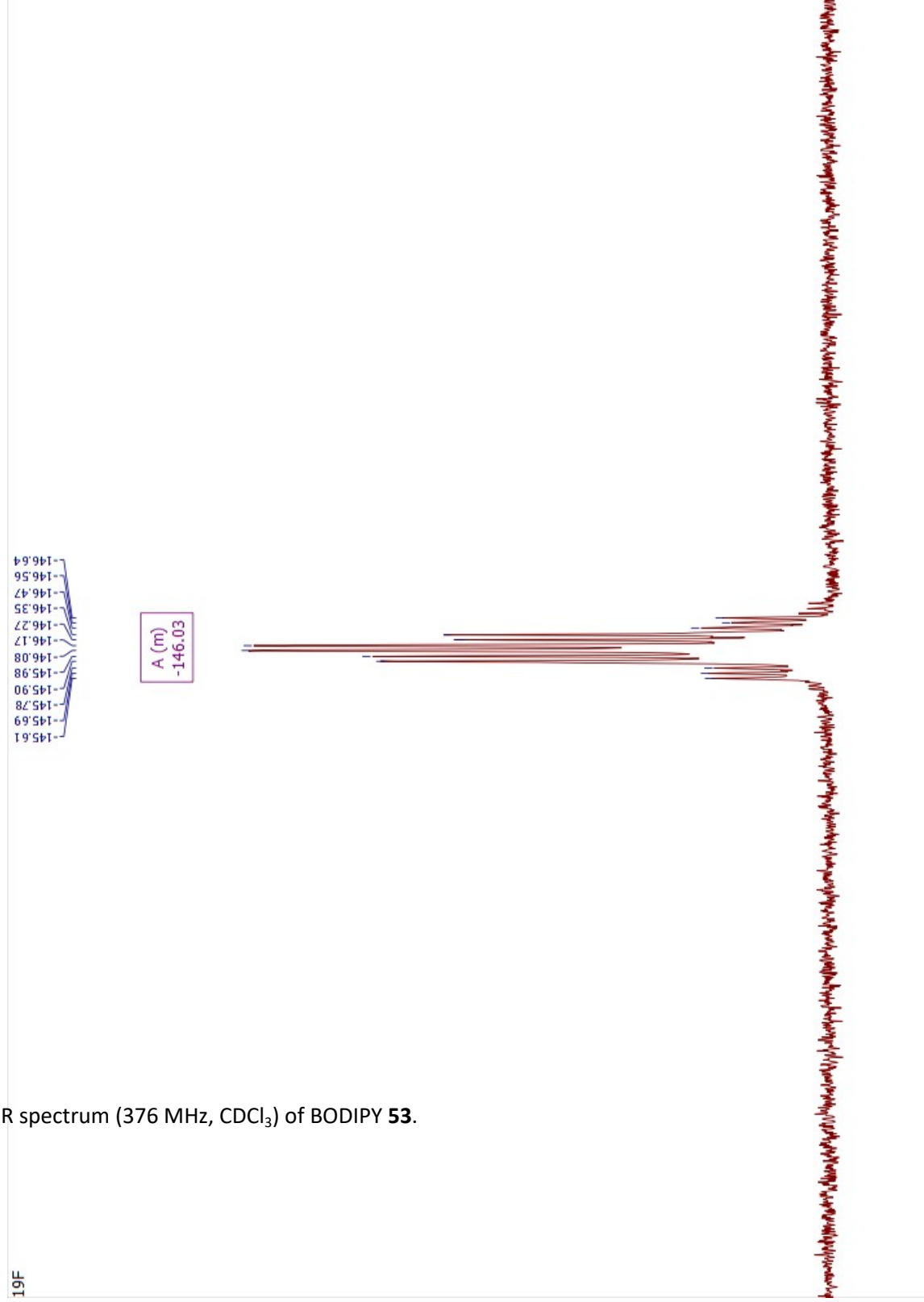
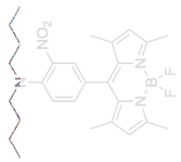


Figure S4.4.3: ^{19}F NMR spectrum (376 MHz, $CDCl_3$) of BODIPY **53**.



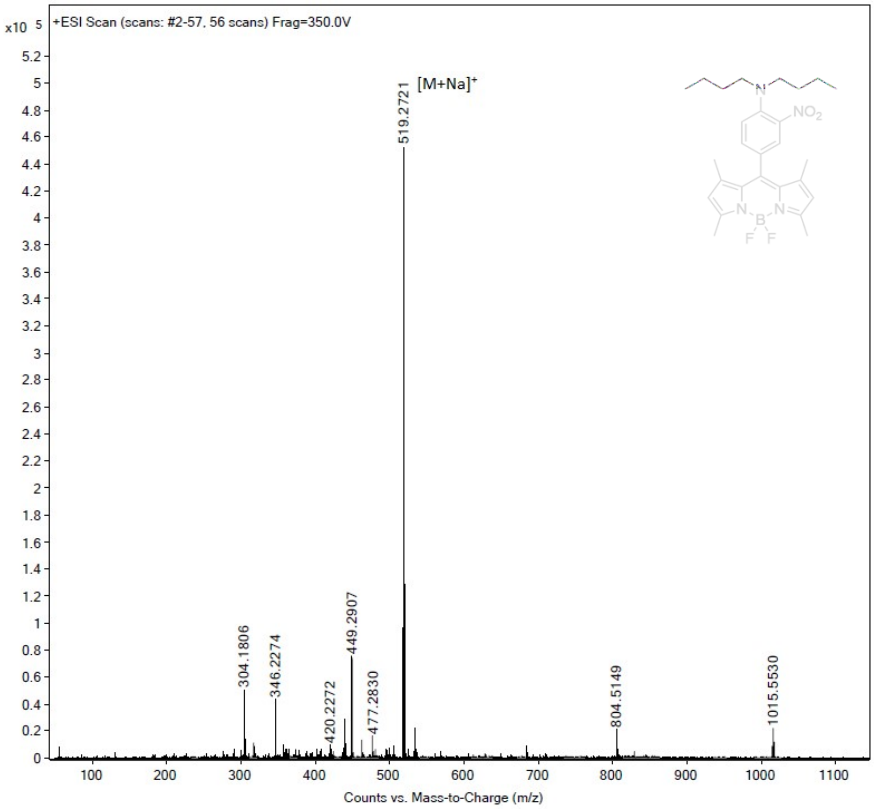


Figure S4.4.4: HRMS spectrum (ESI+) of BODIPY **53**.

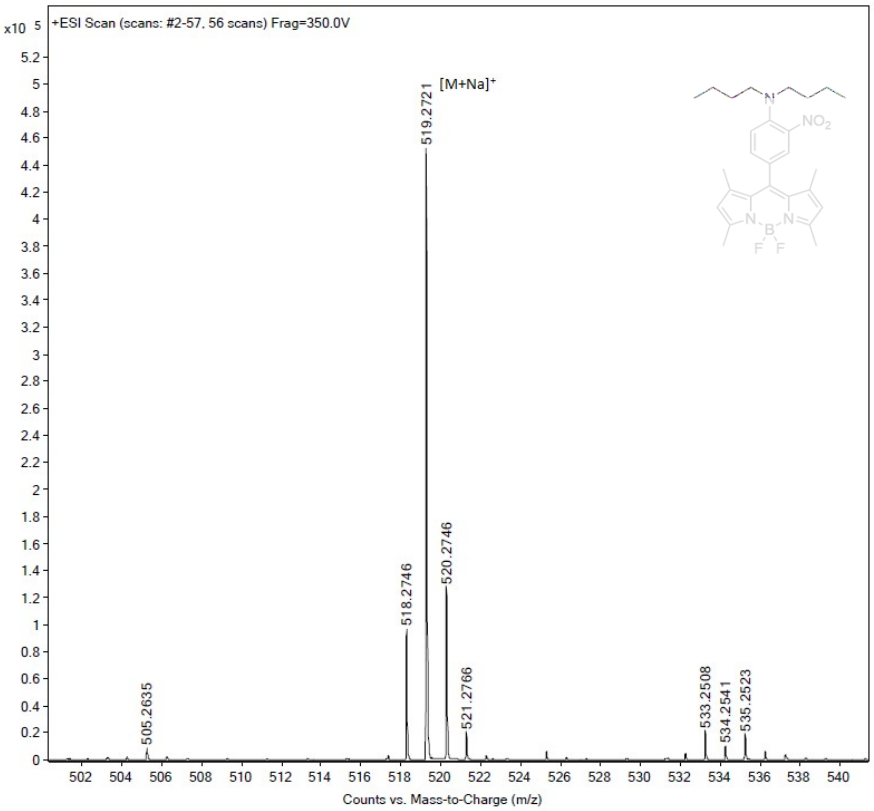


Figure S4.4.5: HRMS spectrum (ESI+) of BODIPY **53**.

S5 Bromination of tetramethyl-BODIPYs (54 – 55)

S5.1 General synthetic procedure

The corresponding 1,3,5,7-tetramethyl substituted BODIPY (1 equiv.) was dissolved in 2 mL of HFIP, NBS (2.5 equiv.) was added, and the mixture was stirred for 1 min at rt. Afterwards, the reaction mixture was diluted with EtOAc and washed with water several times. The organic layer was dried with Na_2SO_4, filtered, and evaporated to dryness. The crude product was purified by column chromatography; the main fraction was collected and evaporated to dryness.

S5.2 2,6-Dibromo-8-[3-nitro-4-(*N*-2-prop-2-enylamino)phenyl]-1,3,5,7-tetramethyl-4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene (**54**):

BODIPY **54** was prepared according to the general synthetic procedure. BODIPY **51** (87 mg, 0.21 mmol) and NBS (91 mg, 0.51 mmol) were dissolved in HFIP. After purification (column chromatography, silica gel, EtOAc/*n*-hexane = 1/9, v/v); BODIPY **54** was obtained as a red solid (53 mg, 92 µmol, 44%). Despite thorough chromatographic purification, this compound still shows tiny signals from an impurity in the range of 3.75 – 4.00 ppm and at 8.25 ppm.

^{1}H NMR (500 MHz, THF-d_8): δ (ppm) = 1.58 (m, 6H, Me), 2.56 (s, 6H, Me), 4.12–4.15 (m, 2H, CH_2), 5.23 (ddd, *J* = 10.4, 1.5 Hz, 1H, **H_2C**=CH-), 5.31 (ddd, *J* = 17.2, 1.6 Hz, 1H, **H_2C**=CH-), 6.01 (ddt, *J* = 17.2, 10.1, 4.9 Hz, 1H, H_2C=**CH**-), 7.20 (d, *J* = 8.8 Hz, 1H, Ar-H_{meta}), 7.41 (dd, *J* = 8.8, 2.1 Hz, 1H, Ar-H_{ortho}), 8.19 (d, *J* = 2.2 Hz, 1H, Ar-H_{ortho}), 8.45 (t, *J* = 5.9 Hz, 1H, NH).

^{13}C NMR (126 MHz, THF-d_8): δ (ppm) = 13.9 (Me), 14.8 (Me), 46.1 (CH_2), 112.3 (C_{Br}), 116.9 (**H_2C**=CH-), 117.0 (Ar-C_{meta}), 121.5*, 127.7 (Ar-C_{ortho}), 131.99 ($C_{Pyrrole}$), 133.5*, 134.9 (H_2C=**CH**-), 136.4 (Ar-C_{ortho}), 141.1 ($C_{Pyrrole}$), 142.1 (C_{meso}), 146.8 (Ar-C_{para}), 154.9 ($C_{Pyrrole}$). *These signals could not be assigned exactly to corresponding carbon atoms. They belong to the Ar-C_{ipso} and the Ar-C_{nitro} of the aryl moiety.

^{19}F NMR (376 MHz, THF-d_8): δ (ppm) = -146.56 – -146.27 (m_c, 2F, BF_2).

HRMS (ESI-TOF, MeOH): *m/z* calcd. for $C_{22}H_{21}BBr_2FN_4O_2^+$ $[M-F]^+$: 563.0082, found: 563.0083, *m/z* calcd. for $C_{22}H_{21}BBr_2F_2N_4O_2Na^+$ $[M+Na]^+$: 604.9964, found: 604.9968, *m/z* calcd. for $C_{22}H_{21}BBr_2F_2N_4O_2K^+$ $[M+K]^+$: 620.9703, found: 620.9707, *m/z* calcd. for $C_{44}H_{42}B_2Br_4F_4N_8O_4Na^+$ $[2M+Na]^+$: 1187.0036, found: 1187.0044.

IR (ATR): $\tilde{\nu}$ (cm^{-1}) = 3358 [ν(NH)], 2925 [ν(CH_2)], 2847 [ν(CH)], 1631 [ν(C=N), ν(C=C)], 1535 [ν_{as}(NO_2)], 1460 [δ(CH_2), δ(Me)], 1346 [ν_{sym}(NO_2)], 1170 and 1119 [ν(BF), ν(CBr)], 755 [δ(HC=CH)].

UV/vis (DCM): λ_{max} (nm) [log (ε/L mol^{-1} cm^{-1})] = 399 [4.17], 528 [4.85].

Fluorescence (DCM): λ_{max} (nm) = 550 at $\lambda_{Excitation}$ (nm) = 380, 510

M.P.(°C): 209-214.

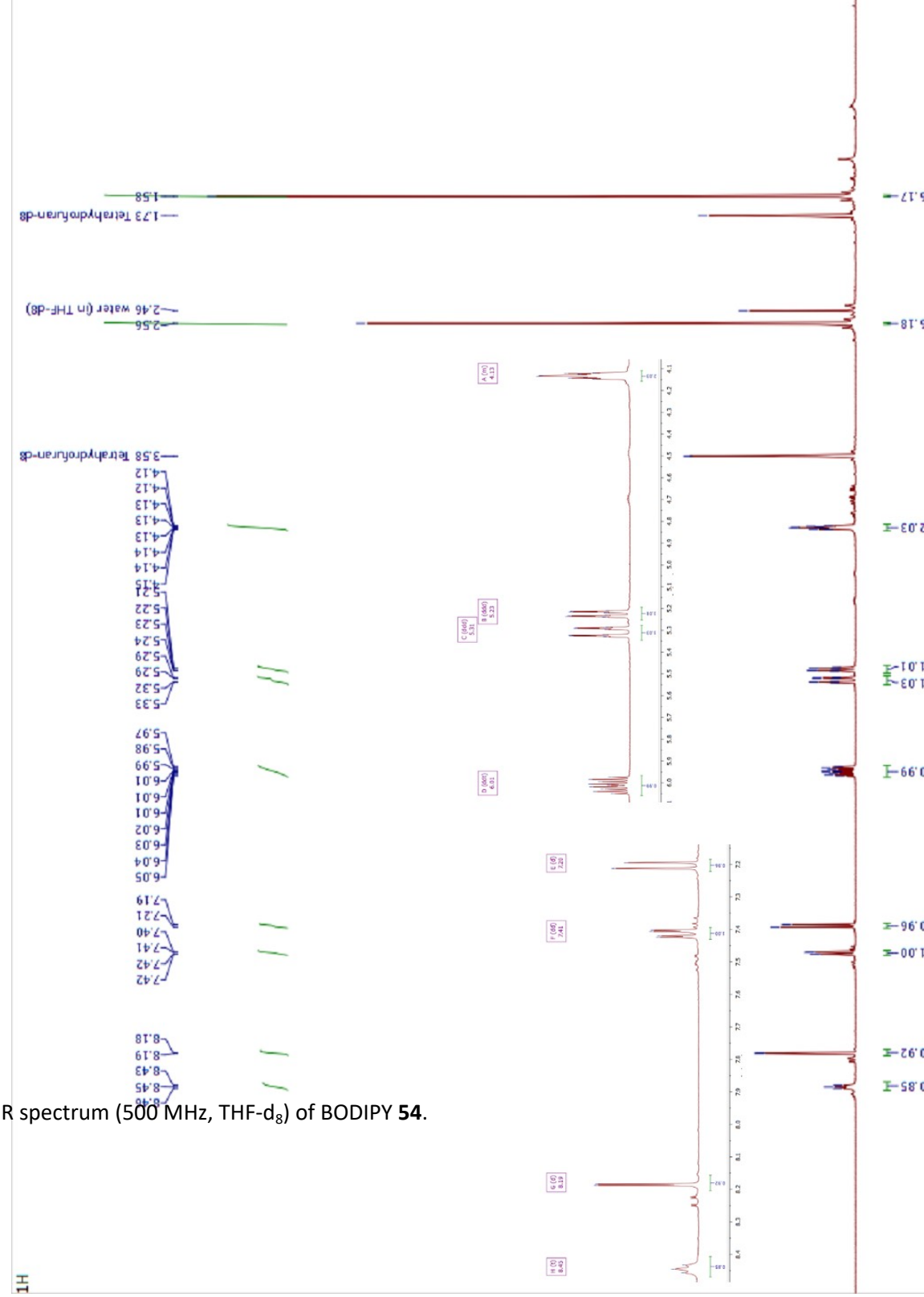
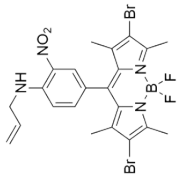


Figure S5.2.1: ^{1}H NMR spectrum (500 MHz, THF-d_8) of BODIPY **54**.



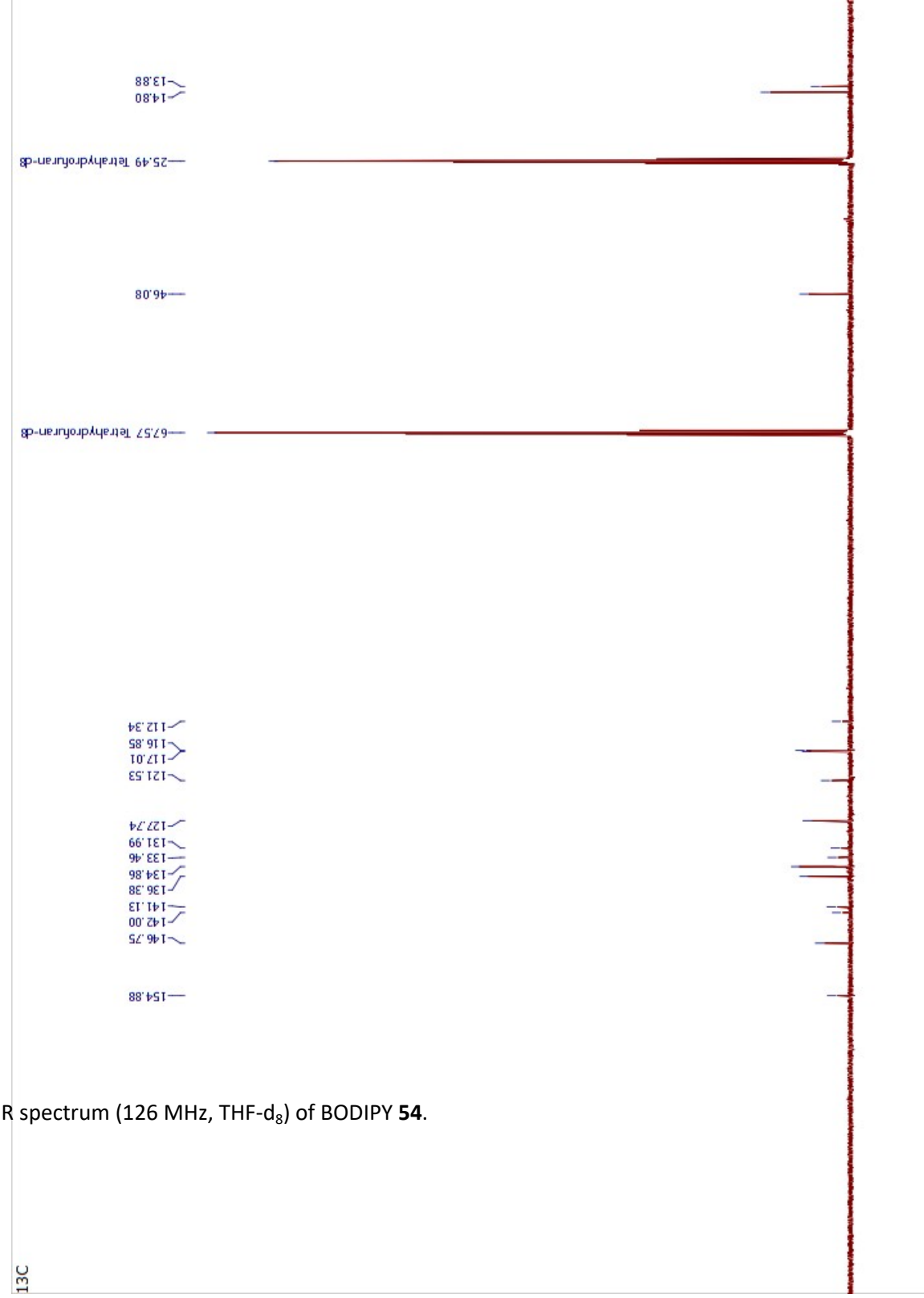
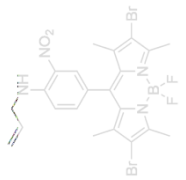


Figure S5.2.2: ^{13}C NMR spectrum (126 MHz, THF-d_8) of BODIPY **54**.



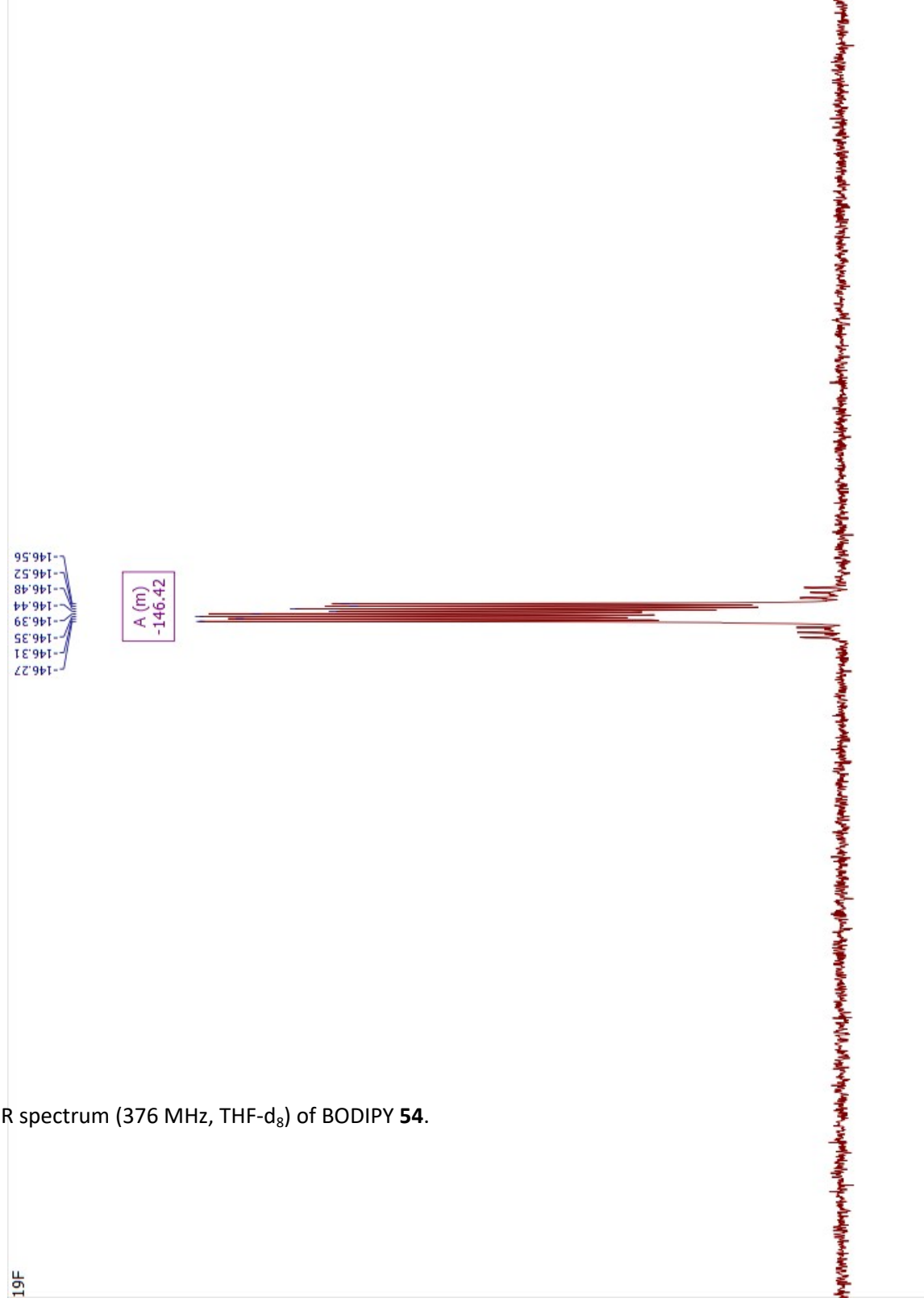
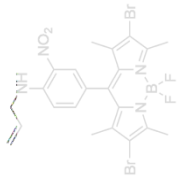


Figure S5.2.3: ^{19}F NMR spectrum (376 MHz, THF-d_8) of BODIPY **54**.



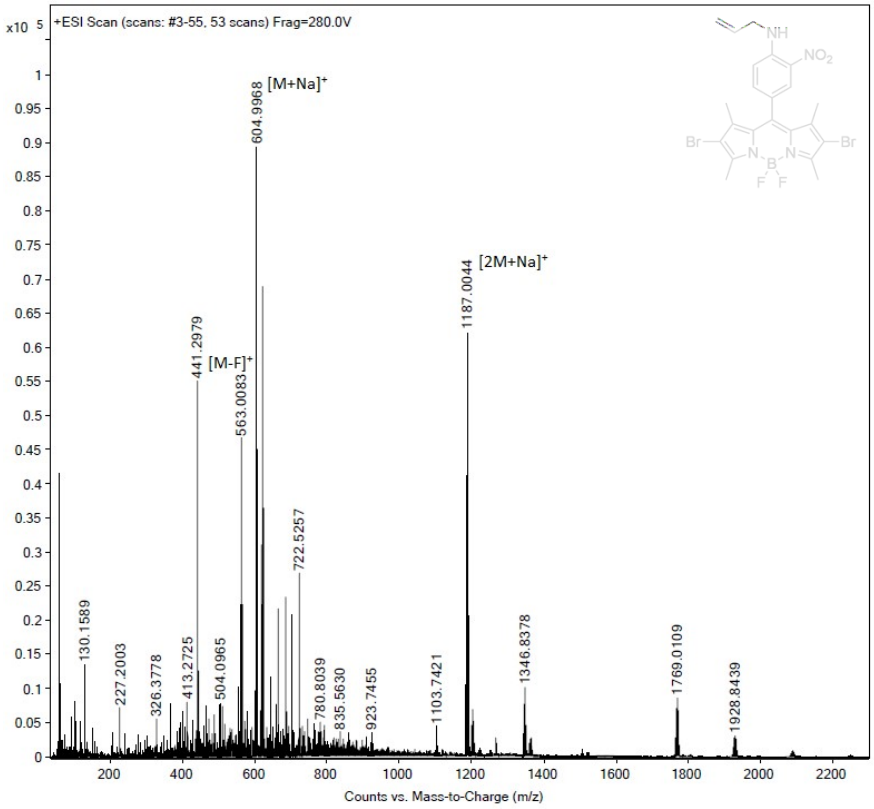


Figure S5.2.4: HRMS spectrum (ESI+) of BODIPY **54**.

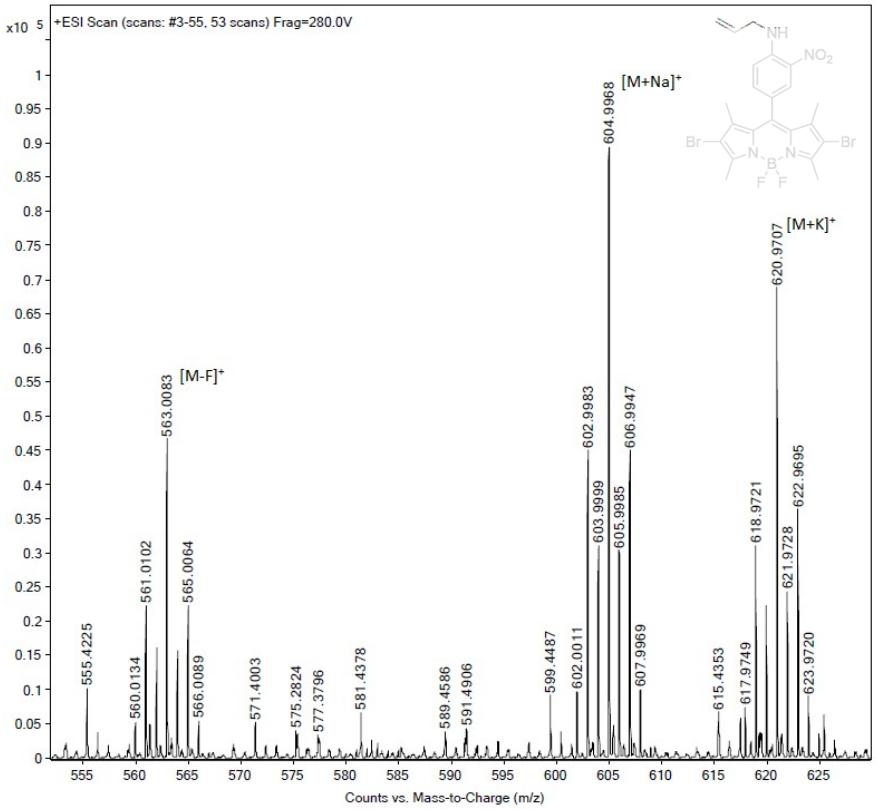


Figure S5.2.5: HRMS spectrum (ESI+) of BODIPY **54**.

S5.3 2,6-Dibromo-8-[4-(*N*-6-methoxy-6-oxohexylamino)-3-nitrophenyl]-1,3,5,7-tetramethyl-4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene (**55**):

BODIPY **55** was prepared according to the general synthetic procedure. BODIPY **52** (95 mg, 0.19 mmol) and NBS (83 mg, 0.46 mmol) were dissolved in HFIP. After purification (column chromatography, silica gel, EtOAc/*n*-hexane = 1/9, v/v); BODIPY **55** was obtained as a red solid (67 mg, 0.10 mmol, 54%). After purification, the ^{1}H-NMR spectrum still showed tiny amounts of *n*-hexane resulting from chromatography.

^{1}H NMR (500 MHz, THF-d_8): δ (ppm) = 1.48–1.54 (m, 2H, CH_2), 1.59 (s, 6H, Me), 1.67–1.71 (m, 2H, CH_2), 1.76–1.82 (m, 2H, CH_2), 2.33 (t, J = 7.3 Hz, 2H, CH_2), 2.56 (s, 6H, Me), 3.46 (td, J = 7.3, 5.6 Hz, 2H, CH_2), 3.60 (s, 3H, Me_{COOMe}), 7.27 (d, J = 8.8 Hz, 1H, Ar-H_{meta}), 7.43 (dd, J = 8.8, 2.2 Hz, 1H, Ar-H_{ortho}), 8.18 (d, J = 2.1 Hz, 1H, Ar-H_{ortho}), 8.27 (t, J = 5.5 Hz, 1H, NH).

^{13}C NMR (126 MHz, THF-d_8): δ (ppm) = 13.9 (Me), 14.9 (Me), 25.98 (CH_2), 27.6 (CH_2), 29.4 (CH_2), 34.3 (CH_2), 43.9 (CH_2), 51.5 (Me_{COOMe}), 112.6 (C_{Br}), 116.5 (Ar-C_{meta}), 121.2*, 127.9 (Ar-C_{ortho}), 132.0 ($C_{Pyrrole}$), 133.2*, 136.6 (Ar-C_{ortho}), 141.1 ($C_{Pyrrole}$), 142.1 (C_{meso}), 146.9 (Ar-C_{para}), 154.9 ($C_{Pyrrole}$), 173.8 (CO). *These signals could not be assigned exactly to corresponding carbon atoms. They belong to the Ar-C_{ipso} and the Ar-C_{nitro} of the aryl moiety.

^{19}F NMR (376 MHz, THF-d_8): δ (ppm) = -146.56 – -146.25 (m_c, 2F, BF_2).

HRMS (ESI-TOF, MeOH): *m/z* calcd. for $C_{26}H_{29}BBr_2F_2N_4O_4Na^+$ $[M+Na]^+$: 693.0488, found: 693.0503, *m/z* calcd. for $C_{26}H_{29}BBr_2F_2N_4O_4K^+$ $[M+K]^+$: 709.0228, found: 709.0235, *m/z* calcd. for $C_{52}H_{58}B_2Br_4F_4N_8O_8Na^+$ $[2M+Na]^+$: 1363.1085, found: 1363.1112.

IR (ATR): $\tilde{\nu}$ (cm^{-1}) = 3379 [ν(NH)], 2926 [ν(CH_2), ν(Me)], 2860 [ν(CH)], 1733 [ν(COOMe)], 1632 [ν(C=N), ν(C=C)], 1532 [ν_{as}(NO_2)], 1457 [δ(CH_2), δ(Me)], 1346 [ν_{sym}(NO_2)], 1163 and 1118 [ν(BF), ν(CBr)], 755 [δ(HC=CH)].

UV/vis (DCM): λ_{max} (nm) [log (ε/L mol^{-1} cm^{-1})] = 404 [4.19], 532 [4.87].

Fluorescence (DCM): λ_{max} (nm) = 550 at $\lambda_{Excitation}$ (nm) = 390, 520.

M.P.(°C): 152-155.

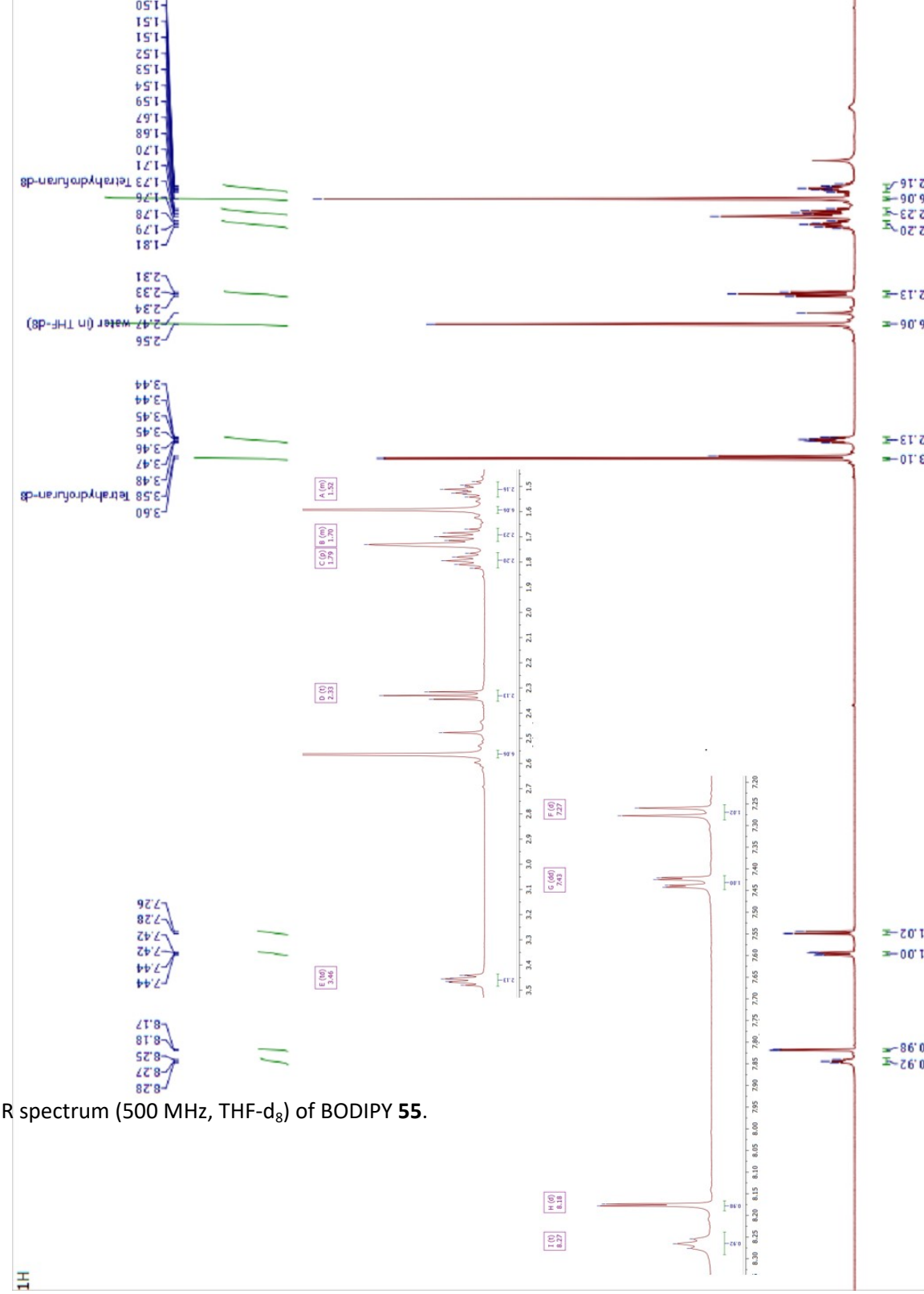
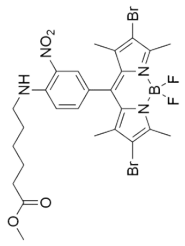


Figure S5.3.1: ^{1}H NMR spectrum (500 MHz, THF-d_8) of BODIPY **55**.



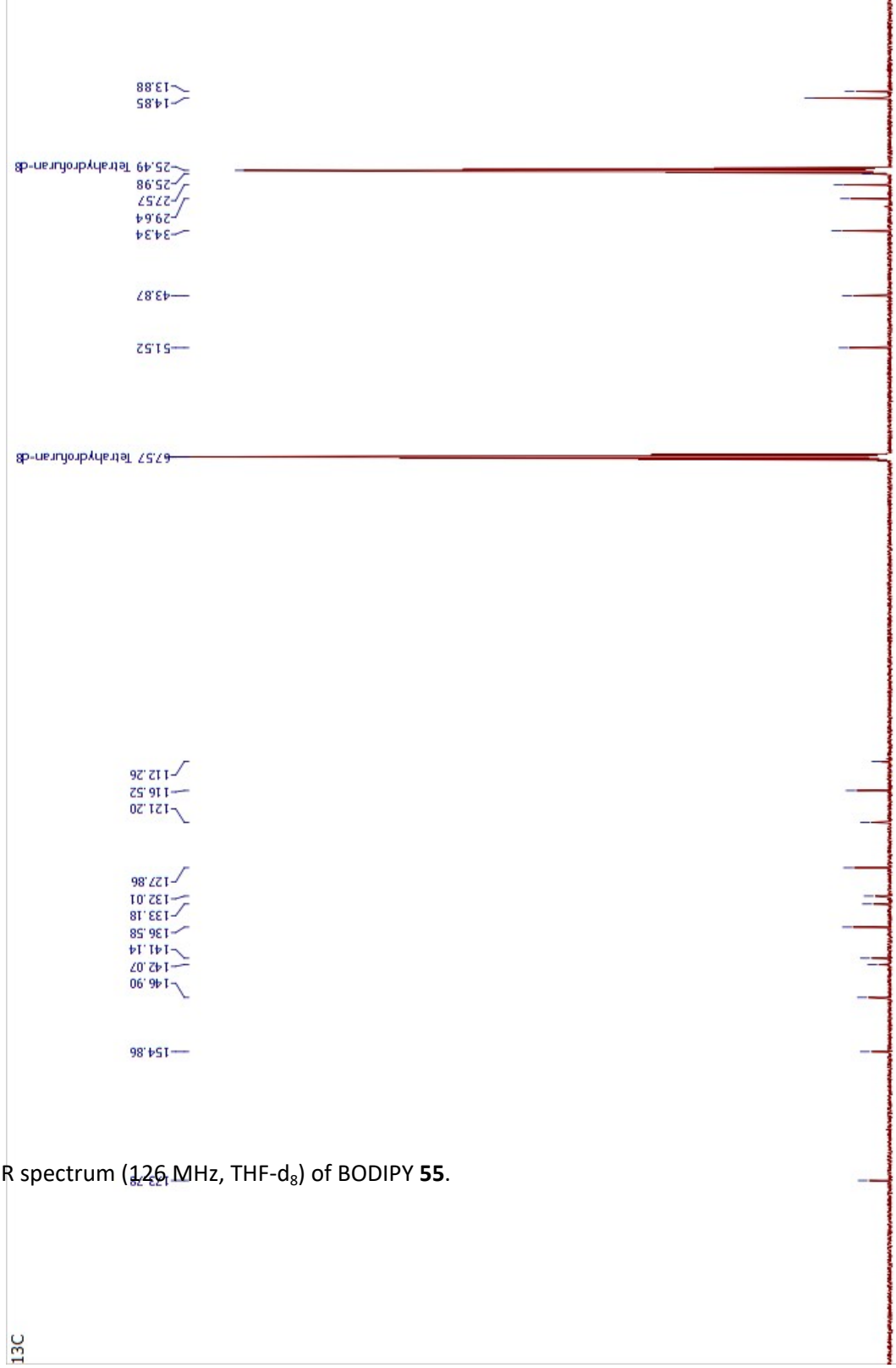


Figure S5.3.2: ^{13}C NMR spectrum (126 MHz, THF-d_8) of BODIPY **55**.

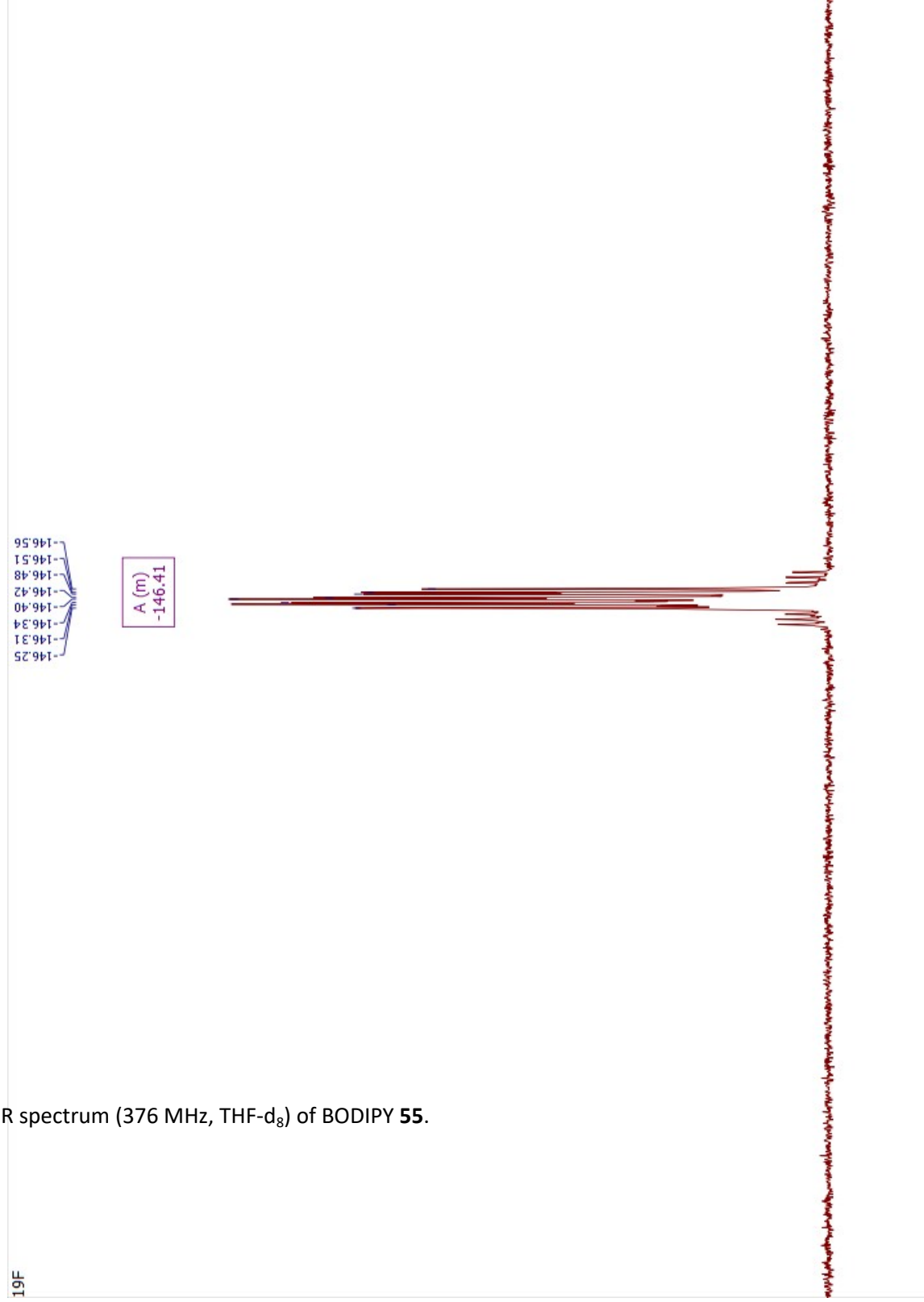


Figure S5.3.3: ^{19}F NMR spectrum (376 MHz, THF-d_8) of BODIPY **55**.

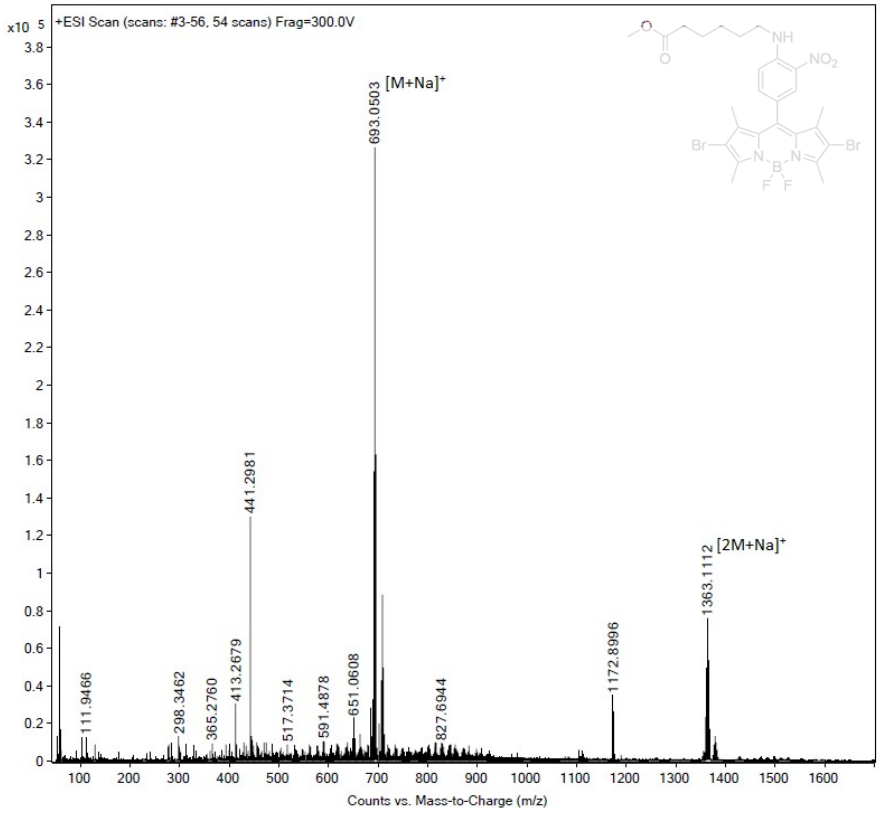


Figure S5.3.4: HRMS spectrum (ESI+) of BODIPY **55**.

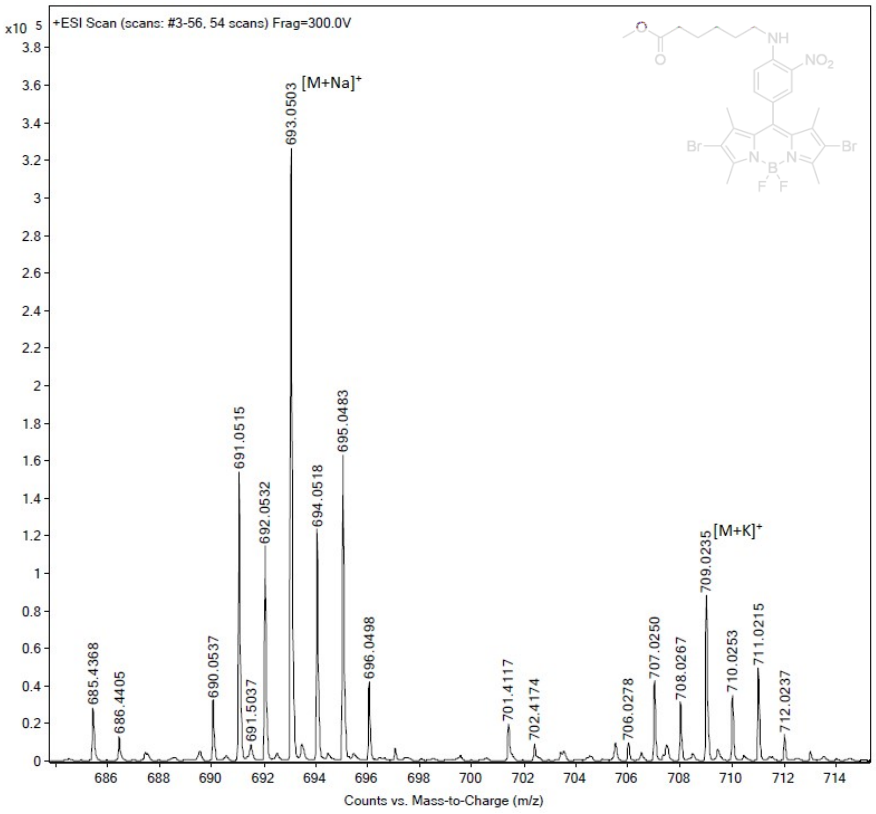


Figure S5.3.5: HRMS spectrum (ESI+) of BODIPY **55**.

S5.4 2,6-Dibromo-8-[4-(*N,N*-dibutylamino)-3-nitrophenyl]-1,3,5,7-tetramethyl-4,4-difluoro-4-bora-3a,4a-diaza-*s*-indacene (**56**):

BODIPY **56** was prepared according to the general synthetic procedure. BODIPY **53** (90 mg, 0.18 mmol) and NBS (80 mg, 0.45 mmol) were dissolved in HFIP. After purification (column chromatography, silica gel, EtOAc/*n*-hexane = 1/9, v/v); BODIPY **56** was obtained as a red solid (68 mg, 0.10 mmol, 57%).

^{1}H NMR (500 MHz, THF-d_8): δ (ppm) = 0.89 (t, J = 7.3 Hz, 6H, Me_{Butyl}), 1.28–1.35 (m, 4H, CH_2), 1.53–1.59 (m, 10H, Me + CH_2), 2.56 (s, 6H, Me), 3.25 (t, J = 7.1 Hz, 4H, CH_2), 7.39 (dd, J = 8.7, 2.0 Hz, 1H, Ar-H_{meta}), 7.46 (dd, J = 8.7, 1.4 Hz, 1H, Ar-H_{ortho}), 7.77 (d, J = 2.0 Hz, 1H, Ar-H_{ortho}).

^{13}C NMR (126 MHz, THF-d_8): δ (ppm) = 13.99 (Me_{Butyl}), 14.3 (Me), 14.6 (Me), 21.0 (CH_2), 30.6 (CH_2), 52.8 (CH_2), 112.3–112.4 (m, C_{Br}), 123.7 (Ar-C_{meta}), 125.4*, 127.2 (Ar-C_{ortho}), 131.8 ($C_{Pyrrole}$), 133.2 (Ar-C_{ortho}), 141.1 ($C_{Pyrrole}$), 141.7 (C_{meso}), 143.9*, 146.4 (Ar-C_{para}), 154.97 ($C_{Pyrrole}$). *These signals could not be assigned exactly to corresponding carbon atoms. They belong to the Ar-C_{ipso} and the Ar-C_{nitro} of the aryl moiety.

^{19}F NMR (376 MHz, THF-d_8): δ (ppm) = -146.52 – -146.31 (m_c, 2F, BF_2).

HRMS (ESI-TOF, MeOH): *m/z* calcd. for $C_{27}H_{34}BBr_2F_2N_4O_2^+$ $[M+H]^+$: 655.1084, found: 655.1087, *m/z* calcd. for $C_{27}H_{33}BBr_2F_2N_4O_2Na^+$ $[M+Na]^+$: 677.0903, found: 677.0899, *m/z* calcd. for $C_{54}H_{66}B_2Br_4F_4N_8O_4Na^+$ $[2M+Na]^+$: 1331.1914, found: 1331.1930.

IR (ATR): $\tilde{\nu}$ (cm^{-1}) = 2957 and 2929 [ν(CH_2), ν(Me)], 2871 [ν(CH)], 1615 [ν(C=N), ν(C=C)], 1533 [ν_{as}(NO_2)], 1461 [δ(CH_2), δ(Me)], 1346 [ν_{sym}(NO_2)], 1171 and 1118 [ν(BF), ν(CBr)], 756 [δ(HC=CH)].

UV/vis (DCM): λ_{max} (nm) [log (ε/L mol^{-1} cm^{-1})] = 389 [4.03], 533 [4.86].

Fluorescence (DCM): λ_{max} (nm) = 551 at $\lambda_{Excitation}$ (nm) = 360, 520.

M.P.(°C): 157-159.

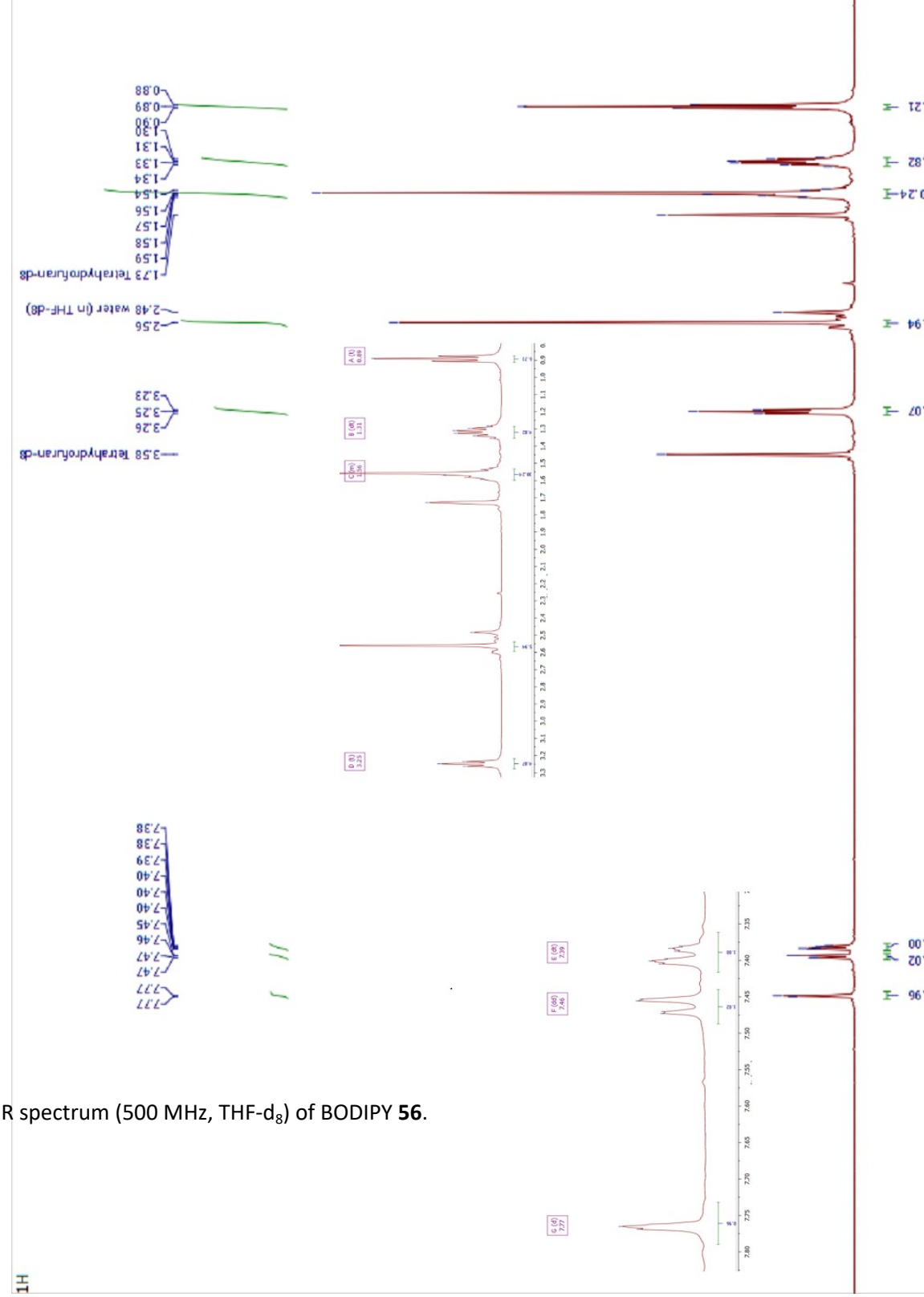
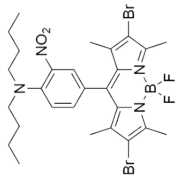


Figure S5.4.1: ^{1}H NMR spectrum (500 MHz, THF-d_8) of BODIPY **56**.



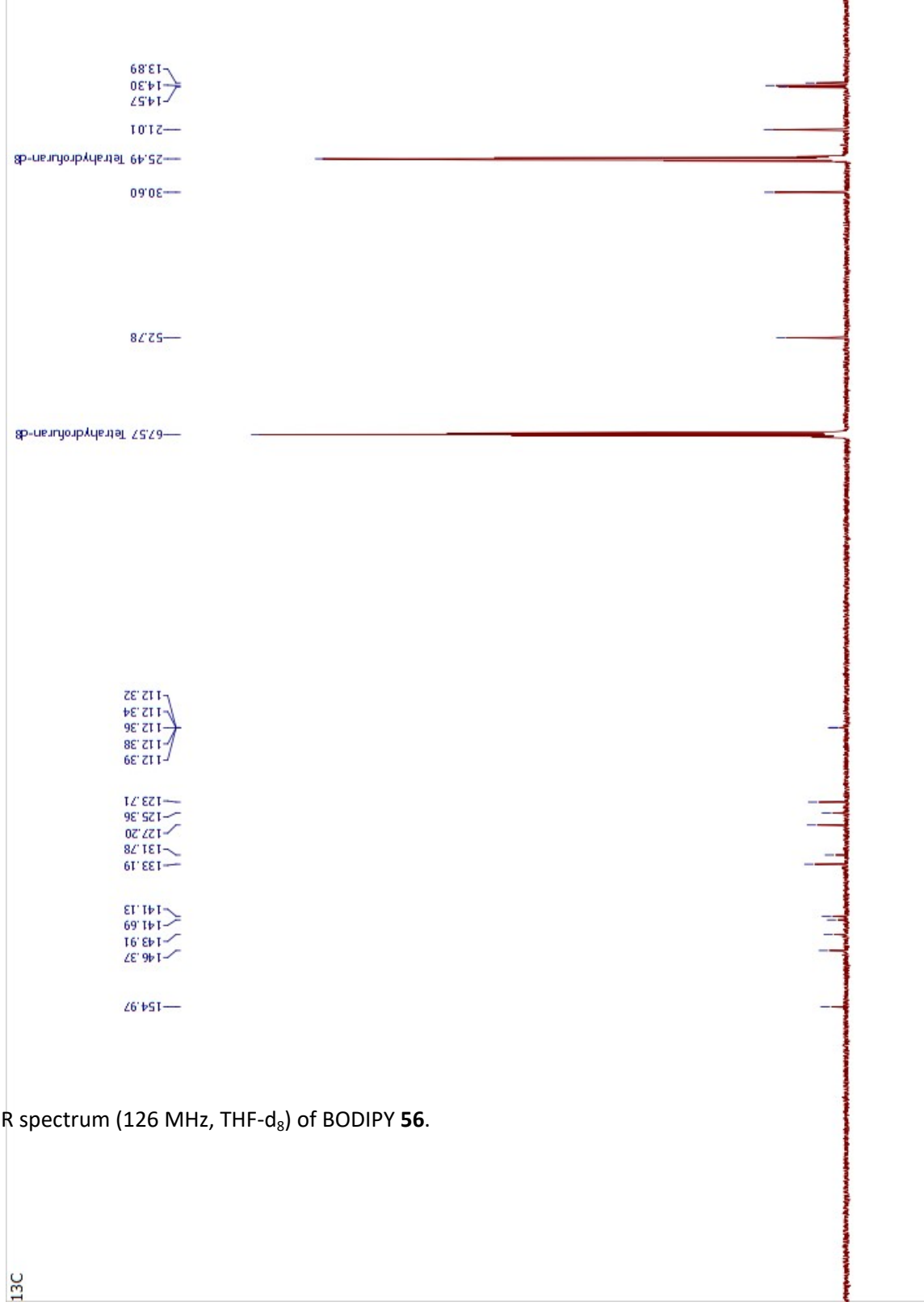
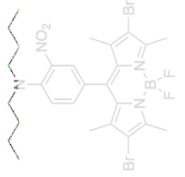


Figure S5.4.1: ^{13}C NMR spectrum (126 MHz, THF-d_8) of BODIPY **56**.



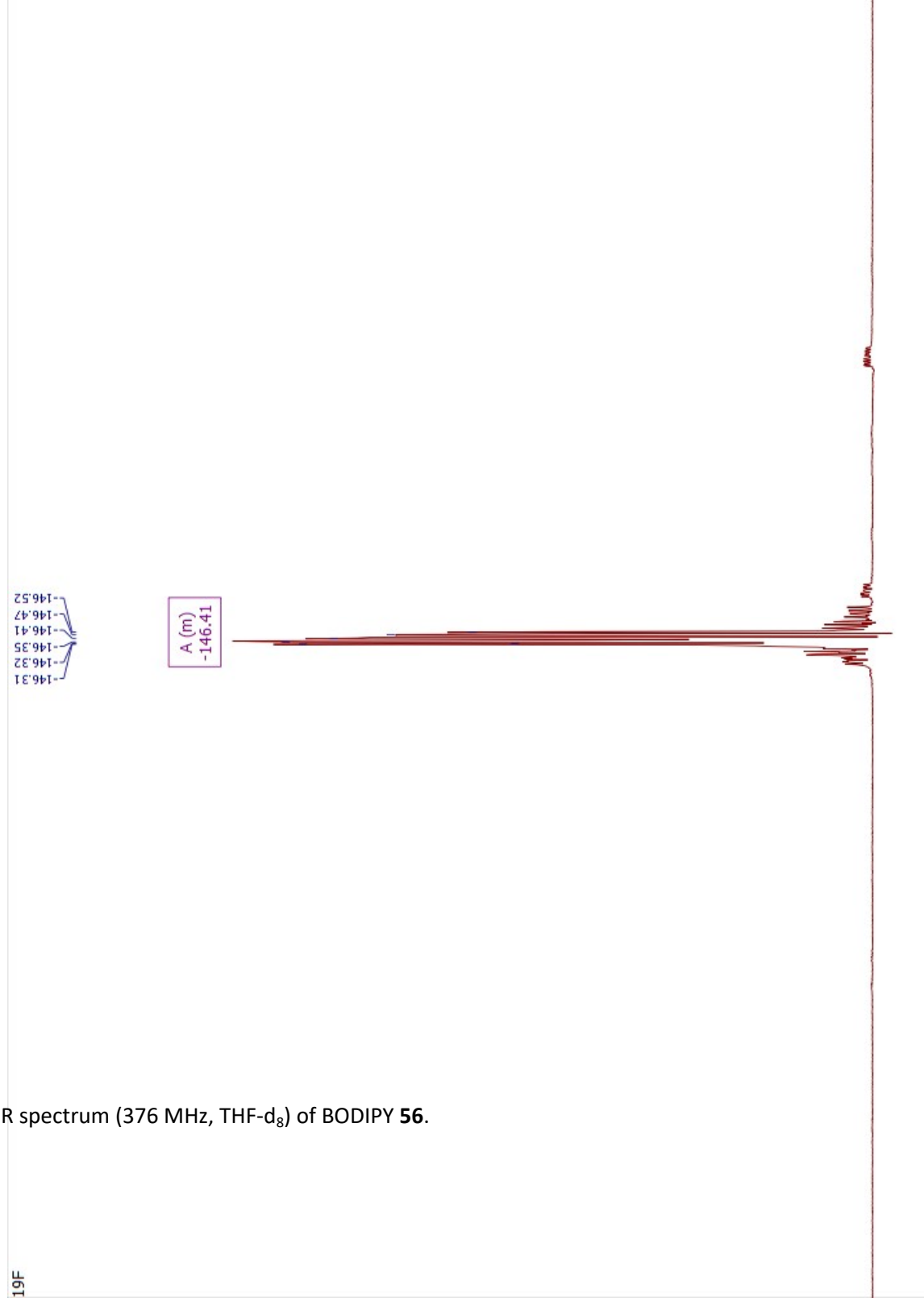
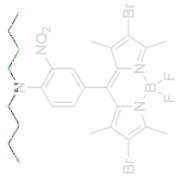


Figure S5.4.3: ^{19}F NMR spectrum (376 MHz, THF-d_8) of BODIPY **56**.



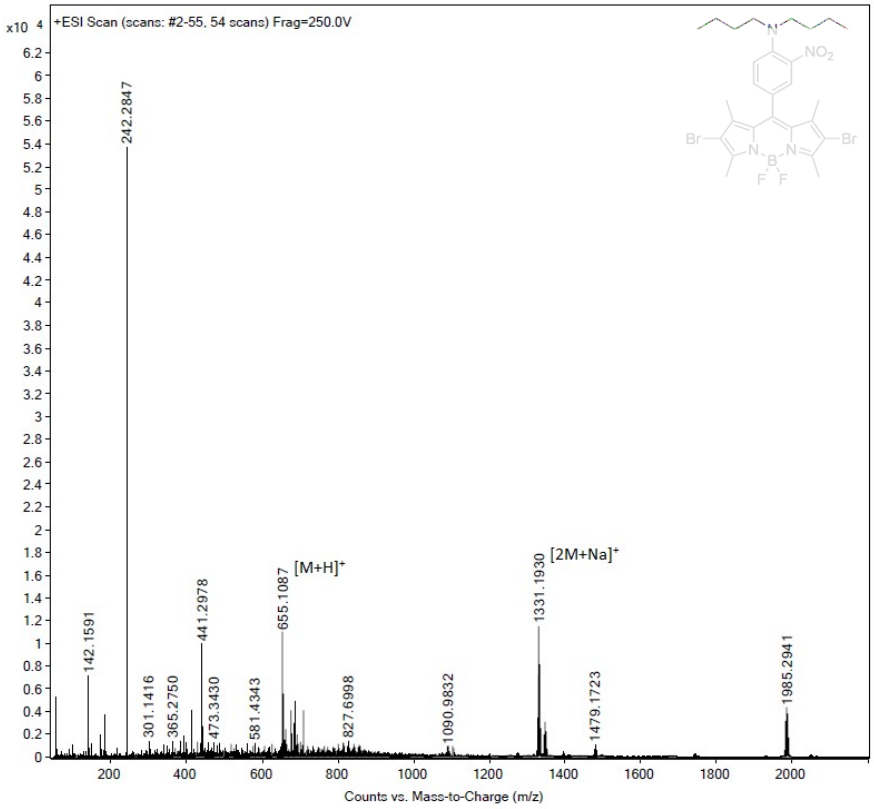


Figure S5.4.4: HRMS spectrum (ESI+) of BODIPY **56**.

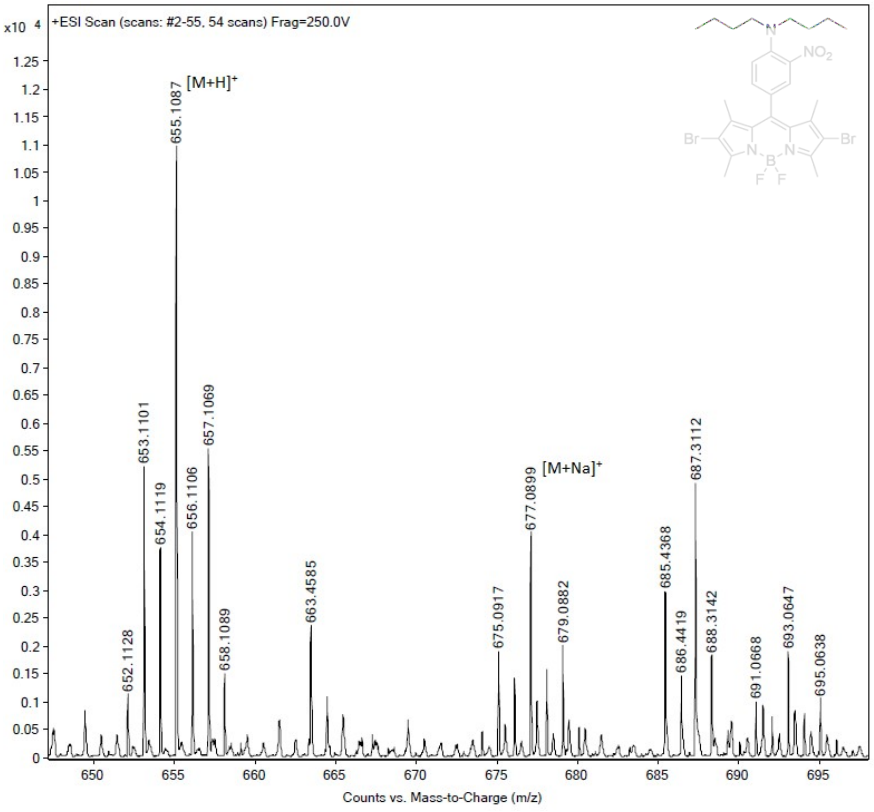


Figure S5.4.5: HRMS spectrum (ESI+) of BODIPY **56**.

S6 References

[1] (a) B. F. Hohlfeld, K. F. Flanagan, N. Kulak, M. O. Senge, M. Christmann and A. Wiehe, *Eur. J. Org. Chem.*, 2019, 4020–4033; (b) C. S. Gutsche, B. F. Hohlfeld, K. J. Flanagan, M. O. Senge, N. Kulak and A. Wiehe, *Eur. J. Org. Chem.*, 2017, 3187–3196; (c) C. S. Gutsche, M. Ortwerth, S. Gräfe, K. J. Flanagan, M. O. Senge, H.-U. Reissig, N. Kulak and A. Wiehe, *Chem. Eur. J.*, 2016, **22**, 13953–13964; (d) H. G. A. Golf, U.-H. Reissig and A. Wiehe, *Org. Lett.*, 2015, **17**, 982–985.

[2] G. Vives, C. Giansante, R. Bofinger, G. Raffy, A. Del Guerzo, B. Kauffmann, P. Batat, G. Jonusauskas, N. D. McClenaghan, *Chem. Commun.* 2011, **47**, 10425-100427.

[3] B. F. Hohlfeld, B. Gitter, K. J. Flanagan, C. J. Kingsbury, N. Kulak, M. O. Senge, A. Wiehe, *Org. Biomol. Chem.* 2020, **18**, 2416-2431.