Supporting Information

BODIPY Phototether Enables Oligonucleotide Cyclization and Subsequent Deprotection by Tissue-Transparent Red Light

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List of abbreviations

CHEX = cyclohexane, DCM = dichloromethane, MeCN = acetonitrile, EtOAc = ethylacetate, TEA = triethylamine, DIPEA *N,N*-diisopropylethylamine, m.p. = melting point, HR-MS = high resolution mass spectrometry, LR-MS = low resolution mass spectrometry, PBS = phosphate buffer saline, DEPC = Diethyl pyrocarbonate, HPA1 = alkyne from amidite hydroxyprolinol, HPA2 = alkyne modification of controlled pore glass solid support, DMT = dimethyltryptamine, CPG = controlled pore glass solid support, TBTA = tris(benzyltriazolylmethyl)amine, HFIP = hexafluoroisopropanol, Pac = phenoxyacetic anhydride, LCAA = long chain alkylamine

General information

The reagents and solvents used in the study were purchased from commercial suppliers and used without purification unless stated otherwise. The solvents were removed on a rotary evaporator at 25–40 °C, and

the products were dried in vacuo overnight. TLC analyses were performed on silica gel-coated aluminum plates (60F254, Merck, Darmstadt, Germany). The compounds were visualized using one of the following methods: exposure to UV light at 254 nm or 365 nm, Flash chromatography purifications were performed on silica gel (40–63 µm, Sigma-Aldrich). Gradient chromatography was performed on an ECOM flash chromatograph. ^{1}H and ^{13}C NMR spectra were measured on a Bruker-400 AVANCE III HD (^{1}H at 400.13 MHz, ^{13}C at 100.62 MHz) in $CDCl_3$ at 300 K. H,H-COSY, spectra were recorded and used for the structural assignment of proton signals. High-resolution mass spectrometry (HR-MS) was performed on an LTQ-orbitrap XL FTMS mass spectrometer (Thermo Fisher Scientific) in electrospray ionisation mode. HPLC-MS was performed on a HPLC-MS-2020 system (Shimadzu Corporation). UV vis spectrometry was measured on an Agilent Cary 8454 UV-vis spectrophotometer (Agilent) and emission spectra were measured on a Duetta fluorescence and absorbance spectrometer (Horiba). Extinction coefficient of oligonucleotide **A** (ε = 183800 M^{-1} cm^{-1}) at 260 nm was calculated from https://eurofinsgenomics.eu/de/ecom/tools/oligo-analysis/ website. Concentration of oligonucleotides **1A** and **A** were determined spectroscopically on NanoDrop 2000 from Thermo Scientific based on the calculated extinction coefficient.

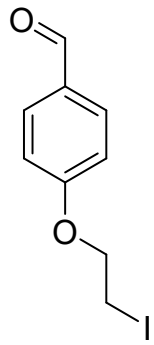
Compound characterization and synthesis

Iodotriphenylphosphonium iodide (PPh_3I_2)

Iodotriphenylphosphonium iodide was prepared according to the literature.[1]

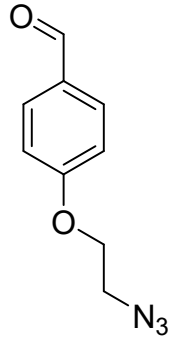
A solution of triphenylphosphine (0.50 g, 0.002 mol, 1. eq) in CH_2Cl_2 (25.0 mL) was added dropwise at 0 °C to iodine (0.48 g, 0.002 mol, 1 eq) in DCM (25.0 mL). The resulting suspension was stirred for 15 min. A complete consumption of triphenylphosphine was detected via TLC (mobile phase = DCM, R_f(product) = 0, R_f(start.mat.) = 0.95). The reaction mixture was concentrated in vacuo and the resulting solid was resuspended in cyclohexane (25.0 mL) and filtered. The combined solids were washed with cyclohexane (100 mL) and dried in vacuo yielding the title compound PPh_3I_2 as an orange solid which was directly used in the next reaction step (0.95 g, 96 % yield).

4-(2-Iodoethoxy)benzaldehyde (3)



Imidazole (154 mg, 2.3 mmol, 1.5 eq) was added to a solution of PPh_3I_2 (854 mg, 1.7 mmol, 1.1 eq) in dry DCM at 0 °C. The mixture turned from orange to yellow after adding imidazole. 4-(2-Hydroxyethoxy)benzaldehyde (250 mg, 1.5 mmol, 1.0 eq) in dry DCM (0.5 mL) was added dropwise. Gradient chromatography was performed using CHEX:EtOAc (0–60 % of EtOAc, *v*/*v*) as the mobile phase, yielding a white solid (317 mg, 76 % yield). **^{1}H NMR** (401 MHz, $CDCl_3$) δ 9.90 (s, 1H), 7.89 – 7.80 (m, 2H), 7.03 – 6.99 (m, 2H), 4.34 (t, J_{HH} = 6.4 Hz, 2H), 3.45 (t, J_{HH} = 6.4 Hz, 2H). **^{13}C NMR** (100.62 MHz, $CDCl_3$) δ 191.20, 163.32, 132.53, 130.92, 115.37, 69.18, 0.51. **HR-MS** (ESI+) m/z calcd. for $C_9H_9IO_2$ [M]+ 275.9647; found 275.9646. **MP**=72–75 °C. 9.90 (s, 1H), 7.89 – 7.80 (m, 2H), 7.03 – 6.99 (m, 2H), 4.34 (t, *J* = 6.4 Hz, 2H), 3.45 (t, 2H).

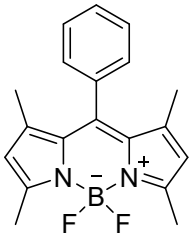
4-(2-Azidoethoxy)benzaldehyde (4)



To a stirred solution of aldehyde **3** (332 mg, 1.2 mmol, 1.0 eq) and K_2CO_3 (498.6 mg, 4 mmol, 3.0 eq) in DMF, NaN_3 (234.5 mg, 4 mmol, 3.0 eq) was slowly added. The reaction mixture was stirred at 50 °C in a heating mantle for 4–5 h. Once the solution was cooled down to room temperature, water was added to dissolve the excess NaN_3, and the solution was extracted with $CHCl_3$ (3 × 50 mL). The combined organic layers were washed 5 times with 50 mL of water to remove DMF and then dried over Na_2SO_4. The excess solvent was distilled off under reduced pressure, producing a colorless oil (178 mg, 77 % yield). **^{1}H NMR** (400.13 MHz, $CDCl_3$): δ_H 9.93 (s, 1H), 7.92 – 7.84 (m, 2H), 7.10 – 7.02 (m, 2H), 4.26 (t, J_{HH} = 5.0 Hz, 2H), 3.68 (t, J_{HH} = 5.0 Hz, 2H) ppm.

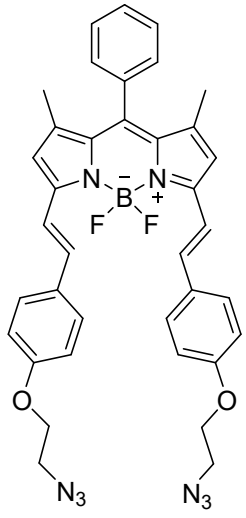
^{1}H NMR spectrum corresponds to the literature.[2]

8-(phenyl)-4,4-difluoro-1,3,5,7-tetramethyl-4H -3aλ^4,4a-diaza- 4λ^4-bora-*s*-indacene (5)



A solution of benzoyl chloride (1.7 mL, 14 mmol, 1.0 eq) in DCM (15.0 mL) was slowly added to a solution of 2,4-dimethyl-1*H*-pyrrole (3.4 mL, 33 mmol, 2.3 eq) in DCM (15 mL) under argon atmosphere. The flask was covered with aluminum foil and heated to 50 °C in a heating mantle for 2h. After cooling down to 0 °C, DIPEA (4.4 mL, 36 mmol, 2.5 eq) was added, followed by BF_3 etherate addition (6.2 mL, 36 mmol, 6.0 eq). The reaction mixture was stirred for 2 h at room temperature. The solution was filtered on SiO_2 and washed with DCM:CHEX (1:1, *v*/*v*). The filtrate was evaporated to dryness, giving orange powder. Gradient chromatography on SiO_2 was performed using CHEX:DCM (0–100 % DCM, *v*/*v*) as a mobile phase. The solvent was removed, yielding an orange powder (815 mg, 18 % yield). **^{1}H NMR** (400.13 MHz, $CDCl_3$) δ 7.46 – 7.35 (m, 3H), 7.24 – 7.19 (m, 2H), 5.91 (s, 2H), 2.48 (s, 6H), 1.30 (s, 6H). **^{1}H NMR** spectrum corresponds to the literature.[3]

3,5-Bis[(*E*)-2-(*p*-(2-Azidoethoxy)phenyl]ethenyl]-4,4-difluoro-8-(phenyl)-1,7-dimethyl-4*H*-3aλ^4,4a-diaza-4λ^4-bora-*s*-indacene (1)



BODIPY **5** (250 mg, 771 mmol, 1 eq) was dissolved in aldehyde **4** (1.5 g, 7.8 mmol, 10.2 eq) in a 30 mL reaction tube. Piperidine (5 drops) was added to initiate the reaction, and the mixture was heated to 60 °C in a heating mantle and left stirring for 8 h. The conversion was monitored by TLC. The reaction mixture was transferred to an extraction funnel with the smallest possible amount of DCM (approximately 10–30 mL) to dissolve the solid. Then, MeOH (200 mL) was added, followed by sodium bisulfite solution (saturated aq., 50 mL). Subsequently, the funnel was thoroughly shaken for 5 min, after which the mixture was diluted with DCM (150 mL) and washed with water (100 mL). The aqueous phase was then washed twice with DCM (50 mL). The combined organic layers were washed twice with water (100 mL) and brine (100 mL). Then, the organic phase was dried over $MgSO_4$ and filtered through cotton, and the solvent was removed under reduced pressure. The reaction product was purified by flash gradient chromatography (CHEX:EtOAc:DCM 100:0:0–40:58:2, *v*/*v*/*v*), the solvent was distilled off under reduced pressure, yielding dark blue crystals (200 mg, 62 % yield).

^{1}H NMR (400.13 MHz, $CDCl_3$) δ 7.65 (s, 1H), 7.63 – 7.55 (m, 4H), 7.53 – 7.46 (m, 3H), 7.39 – 7.30 (m, 3H), 7.21 (d, J_{HH} = 16.3 Hz, 2H), 6.98 – 6.92 (m, 4H), 6.62 (s, 2H), 4.20 (t, J_{HH} = 5.0 Hz, 4H), 3.63 (t, J_{HH} = 5.0 Hz, 4H), 1.44 (s, 6H).
^{13}C NMR (100.62 MHz, $CDCl_3$) δ 159.09, 152.78, 142.06, 138.50, 135.66, 135.38, 133.37, 130.35, 129.22, 129.19, 129.07, 128.64, 117.70, 115.03, 67.15, 50.29, 14.75.
HR-MS (ESI+) m/z calcd. for $C_{37}H_{33}BF_2N_8O_2$ [M]+ 671.2857; found 671.2857.

Oligonucleotide synthesis

Oligonucleotide synthesis was performed according to the literature.[4]

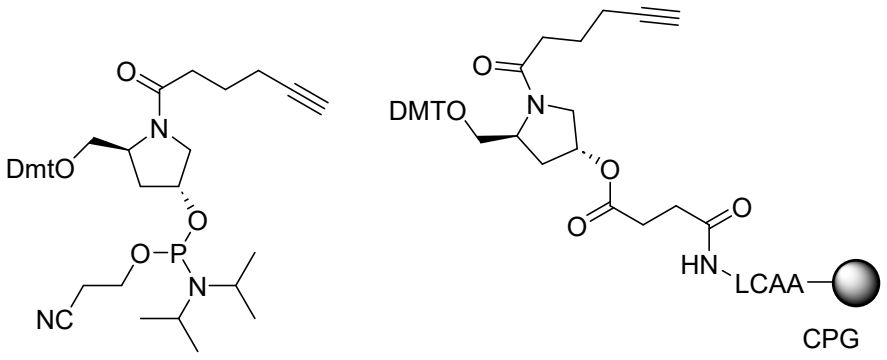
Milli-Q water was treated with 0.1% DEPC overnight and autoclaved before use.

The following oligonucleotide was synthesized by solid-phase synthesis:

A: 5'- HPA2 GCA TAA ATA AAG GTG' HPA1- 3'

The complementary sequence: 5'- CAC CTT TAT TTA TGC 3' was obtained from Biomers.

Phosphoramidites used for the solid-phase synthesis:
G: iPr-Pac-dG-CE phosphoramidite (Linktech) or dG(n-tBPAC) CED phosphoramidite (ChemGenes)
T: DMT-dT phosphoramidite (Sigma Aldrich)
C: DMT-dC(ac) phosphoramidite (Sigma Aldrich)
A: DMT-dA(tac) phosphoramidite (Sigma Aldrich)
HPA1: Alkyne amidite hydroxyprolinol (Lumiprobe)
HPA2: Alkyne modification of CPG (Lumiprobe)



HPA1 **HPA2**

Figure S1. Structures of the alkyne modifiers **HPA1** and **HPA2**.

The solid-phase synthesis of the alkyne-modified DNA oligonucleotide **A** was performed on ABI 392 DNA/RNA synthesizer at a 1-µmol scale. Alkyne-modified CPG of the size 1000 Å (**HPA2**, Lumiprobe) was used as a solid support. The oligonucleotides were synthesized in DMT-on mode under UltraMILD© conditions (Pac-Anhydride/pyridine in tetrahydrofuan as a capping reagent). 0.3 M BTT in acetonitrile (emp Biotech) was used as an activator. The coupling time was 34 s. After deprotection with 32% NH_3 (4 h, 28 °C), the solvent was evaporated under reduced pressure in a vacuum centrifuge at 4 °C, and the

crude product was purified by RP-HPLC on an Agilent 1200 Series Gradient HPLC System equipped with a Waters XBridge Peptide BEH C18 OBD Prep column (300 Å, 5 µm, 10 mm x 250 mm) using *gradient 1* (400 mM HFIP/16.3 mM TEA buffer pH = 7.9, methanol; gradient 1 time plan: 5% MeOH for 2 min, 5% to 25% MeOH in 0.5 min, 25% to 50% MeOH in 6 min, 50% to 100% MeOH in 2 min, flow rate 4 ml/min, 35 °C). After solvent evaporation, the DMT group was removed with 80% acetic acid (20 min, 25 °C), and the oligonucleotide was purified again by RP-HPLC on an Agilent 1200 equipped with a Waters XBridge Peptide BEH C18 OBD Prep column (300 Å, 5 µm, 10 mm x 250 mm) using *gradient 1* (400 mM HFIP/16.3 mM TEA buffer pH = 7.9, methanol; gradient 1 time plan: 5% MeOH for 2 min, 5% to 25% MeOH in 0.5 min, 25% to 50% MeOH in 6 min, 50% to 100% MeOH in 2 min, flow rate 4 ml/min, 35 °C) to obtain 0.26 µmol of **A** (26 % yield).

The purity and identity of **A** were confirmed by LC-MS (LC-system: Agilent 1200 equipped Waters XBridge Peptide BEH C18 column (300 Å, 3.5 µm, 2.1 mm x 250 mm), using *gradient 2* (400 mM HFIP/16.3 mM TEA bufferpH = 7.9,methanol; gradient time plan: 5% MeOH for 5 min, 5% to 40% MeOH in 2 min, 40% to 80% MeOH in 16 min, 80% to 100% MeOH in 5 min, flow rate 0.25 ml/min, 40 °C), MS-system: Bruker micrOTOF-QII ESI.

Oligonucleotide cyclization

For preparation of oligonucleotide **1A**, the following reagents were added to an aqueous solution of oligonucleotide **A** (5 nmol in 2.5 µL Milli-Q water, *c* = 2 mM) in the corresponding order: Phototether **1** (2.5 µL, 5 eq, conc. 10 mM), freshly prepared Cu(I)TBTA-complex (aq., 1.5 µL, *c* = 33.3 mM) and DMSO (0.5 µL) to a final oligonucleotide concentration of 714 µM. The solution of Cu(I)TBTA-complex was freshly prepared by mixing aqueous solutions of CuI (1 eq., 5 µL, *c* = 100 mM) and TBTA (2 eq., 10 µL, *c* = 100 mM) at room temperature and vortexing for 10 s, immediately used for the cyclization reaction. The reaction tube was sealed under argon and shaken for 60 min at 45 °C. The reaction mixture was diluted in 400 mM HFIP/16.3 mM TEA buffer and purified by RP-HPLC on a Agilent 1200 Series Gradient HPLC System equipped with a Waters XBridge Peptide BEH C18 column (300 Å, 3.5 µm, 4.6 mm x 250 mm) using *gradient 5* (400 mM HFIP/16.3 mM TEA buffer pH = 7.9, methanol; gradient 5 time plan: 5% MeOH for 2 min, 5% to 25% MeOH in 8 min, 25% to 40% MeOH in 2 min, 40% to 60 % MeOH in 18 min, 60% to 100% MeOH in 5 min, flow rate 0.7 ml/min, 45 °C), yielding 1.16 mmol of final product **1A** (12% yield).

Absorption and emission spectra

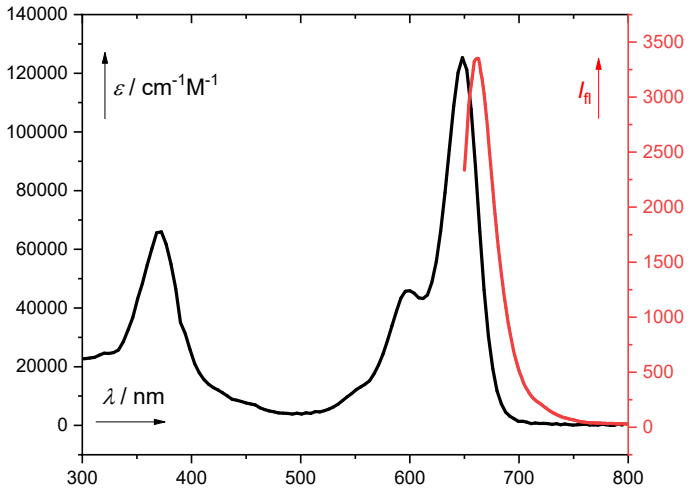


Figure S2. Cyclized oligonucleotide **1A** in PBS: Absorption spectrum (black), normalized emission spectrum (red), exc. wavelength 625 nm, $c \approx 3 \cdot 10^{-5}$ M.

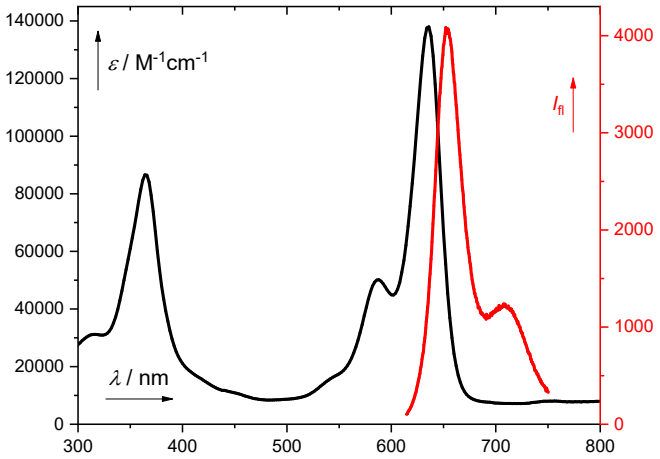


Figure S3. BODIPY phototether **1** in DCM:MeOH (1:9, v/v): Absorption spectrum (black), normalized emission spectrum (red), exc. wavelength 540 nm, $c \approx 3 \cdot 10^{-5}$ M.

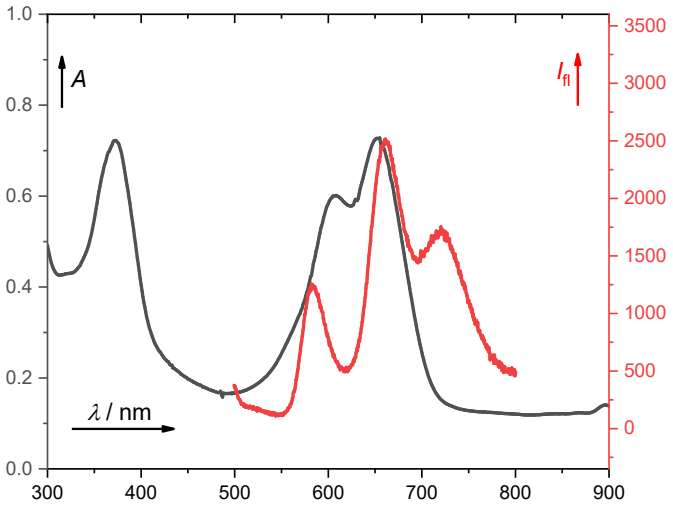


Figure S4. BODIPY phototether **1** in DMSO:water (1:1, v/v): Absorption spectrum (black), normalized emission spectrum (red), exc. wavelength 400 nm, *c* [M] not determined due to aggregation.

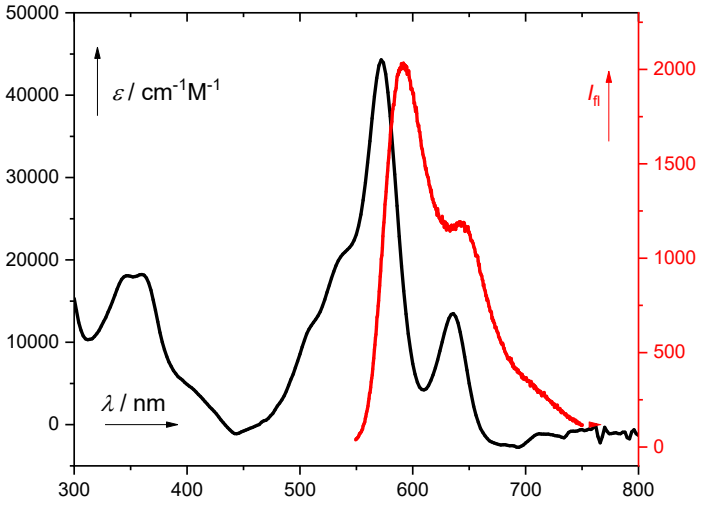


Figure S5. BODIPY **1A-irr** after irradiation with 617-nm LED in PBS: Absorption spectrum (black), normalized emission spectrum (red), exc. wavelength 540 nm, $c \approx 3 \cdot 10^{-5}$ M.

Irradiation and actinometry

The linker **1** was irradiated with a 625-nm LED in the mixture of DCM/MeOH (9/1, v/v, Figure S7) and DMSO/water ($c \approx 6 \cdot 10^{-6}$ M, Figure S6). The cyclized oligonucleotide **1A** was irradiated with a 617-nm LED in aerated and oxygen-saturated PBS buffer (Figure S9). The photon fluxes of the 617-nm and 625-nm LED (~$1 \cdot 10^{-6}$ Einstein s^{-1}) were determined using a trifluoromethyl fulgide actinometer[5] and the photoreaction quantum yields were calculated using these values. The photoreaction was monitored by absorption spectroscopy following the absorption decay at 650 nm. The quantum yield of photorelease of the linker **1** was measured in the mixture of DCM/MeOH (9/1, v/v) and the quantum yield of the cyclized oligonucleotide **1A** ($c \approx 1.3 \cdot 10^{-5}$ M) was measured in aerated and oxygen-saturated PBS buffer, respectively.

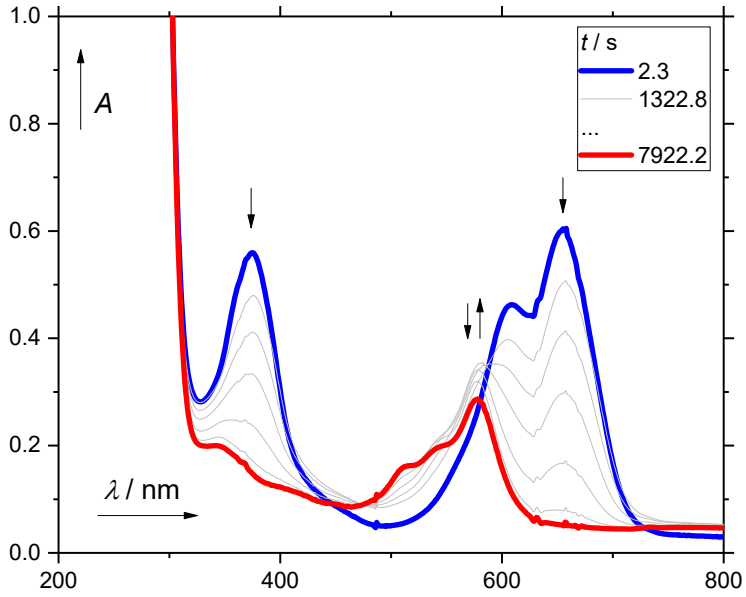


Figure S6. Photolysis of BODIPY linker **1** in a DMSO:water (1:1, *v*/*v*, $c \approx 3 \cdot 10^{-5}$ M) mixture, irradiation with 625-nm LED; absorption spectra.

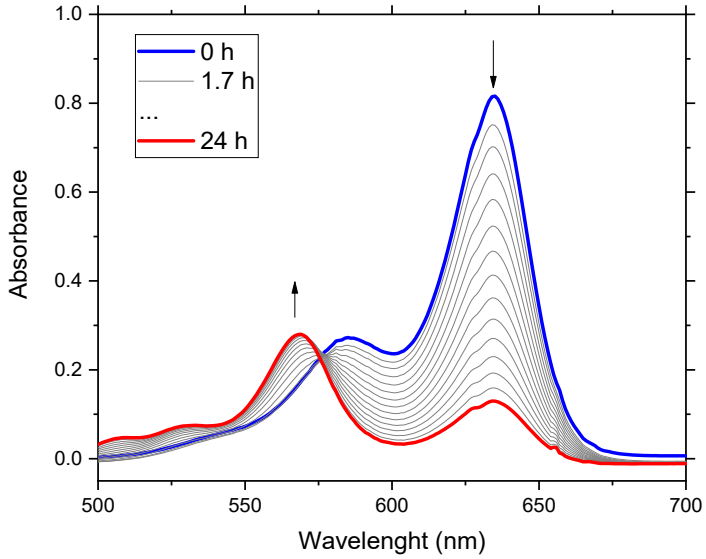


Figure S7. Photolysis of BODIPY linker **1** in a DCM:MeOH (1:9, *v*/*v*, $c \approx 6 \cdot 10^{-6}$ M) mixture, irradiation with 625-nm LED; absorption spectra.

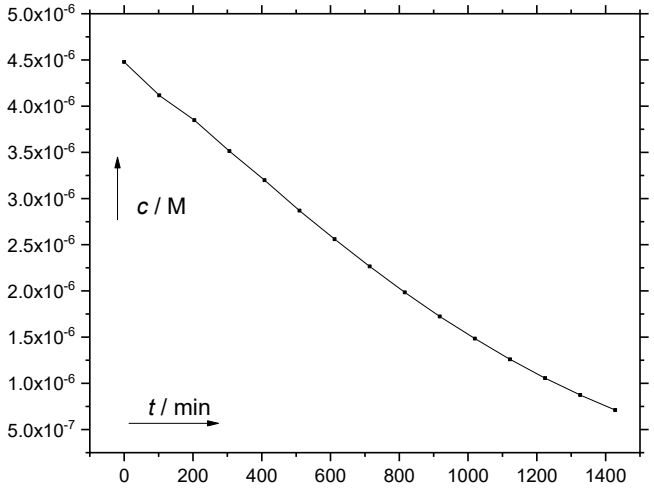


Figure S8. Photolysis of BODIPY linker **1** in a DCM:MeOH (1:9, *v*/*v*, $c \approx 6 \cdot 10^{-6}$ M) mixture, irradiation with 625-nm LED; variation of concentration as a function of time.

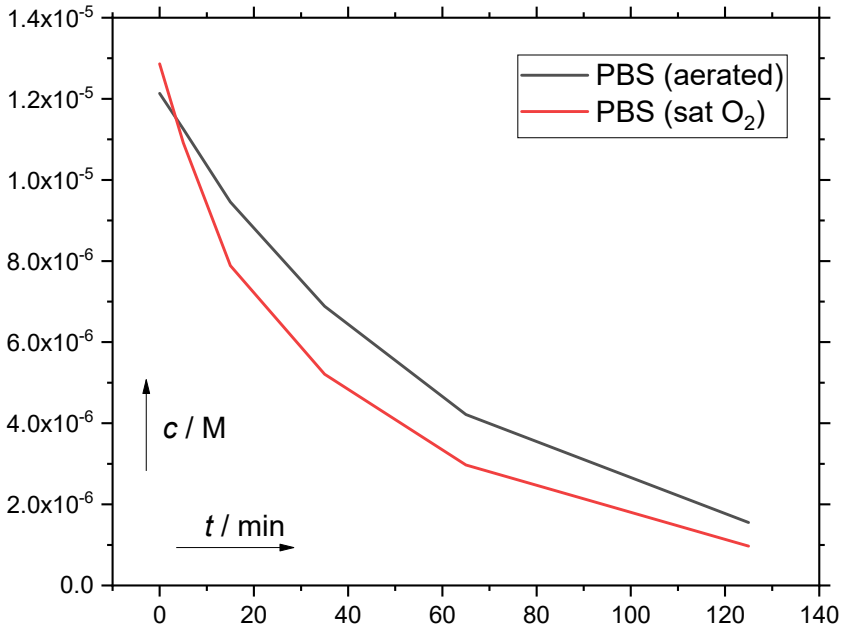


Figure S9. Photolysis of **1A** irradiated with 617-nm LED in aerated PBS (black) and oxygenated PBS (red). Dependence of the concentration of **1A** on irradiation time.

LC-MS-analysis

Table 1 MS data of oligonucleotides (ESI- ionization)

ID	calculated [Da]	found [Da]
A	5193	5194
1A	5863	5862
1A-irr	5895	5894

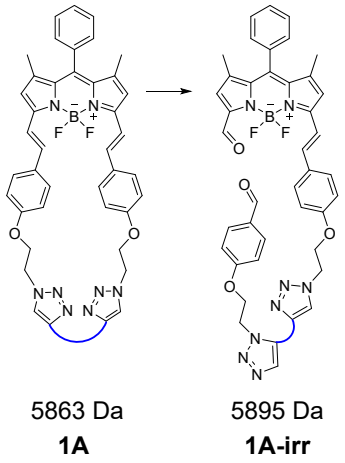


Figure S10. Molar masses of **1A** and its photolysis product **1A-irr** (the blue curved line corresponds to the oligonucleotide **A**)**.**

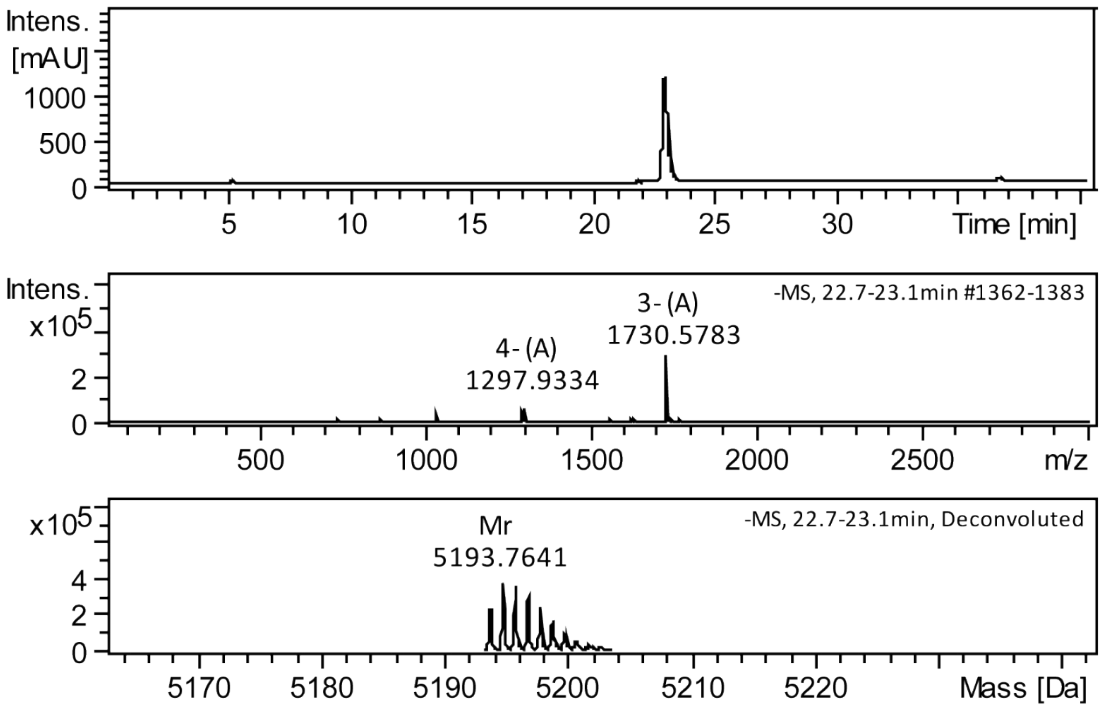


Figure S11. LC-MS chromatogram of the purified oligonucleotide **A.**

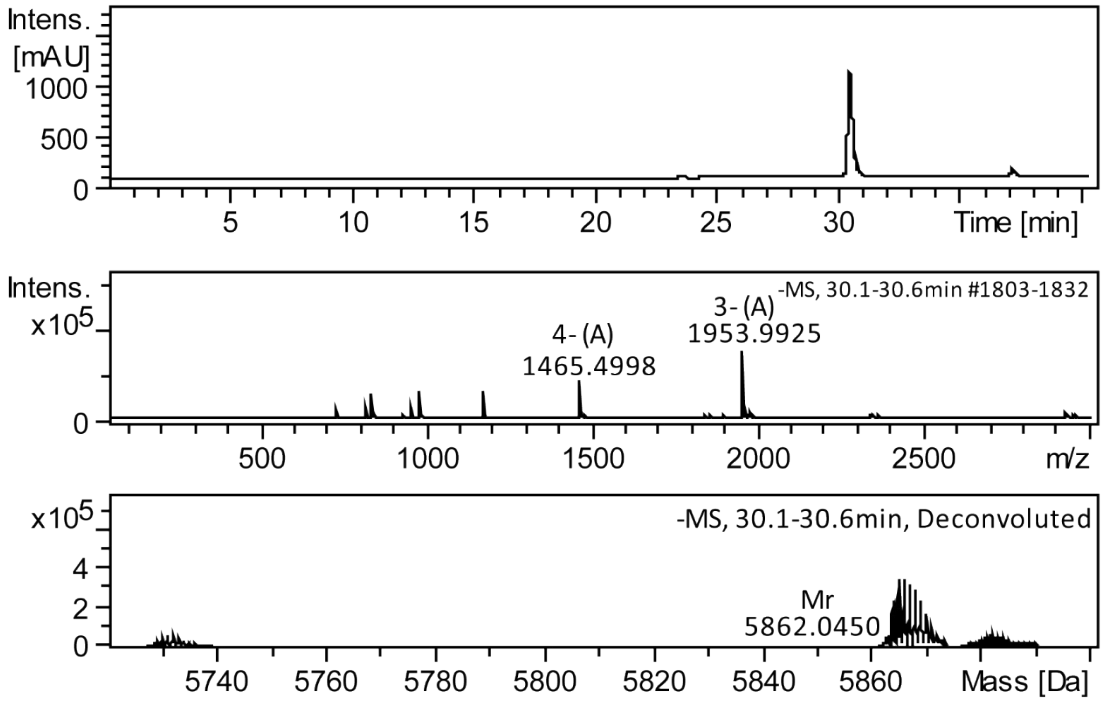


Figure S12. LC-MS chromatogram of the purified cyclized oligonucleotide **1A.**

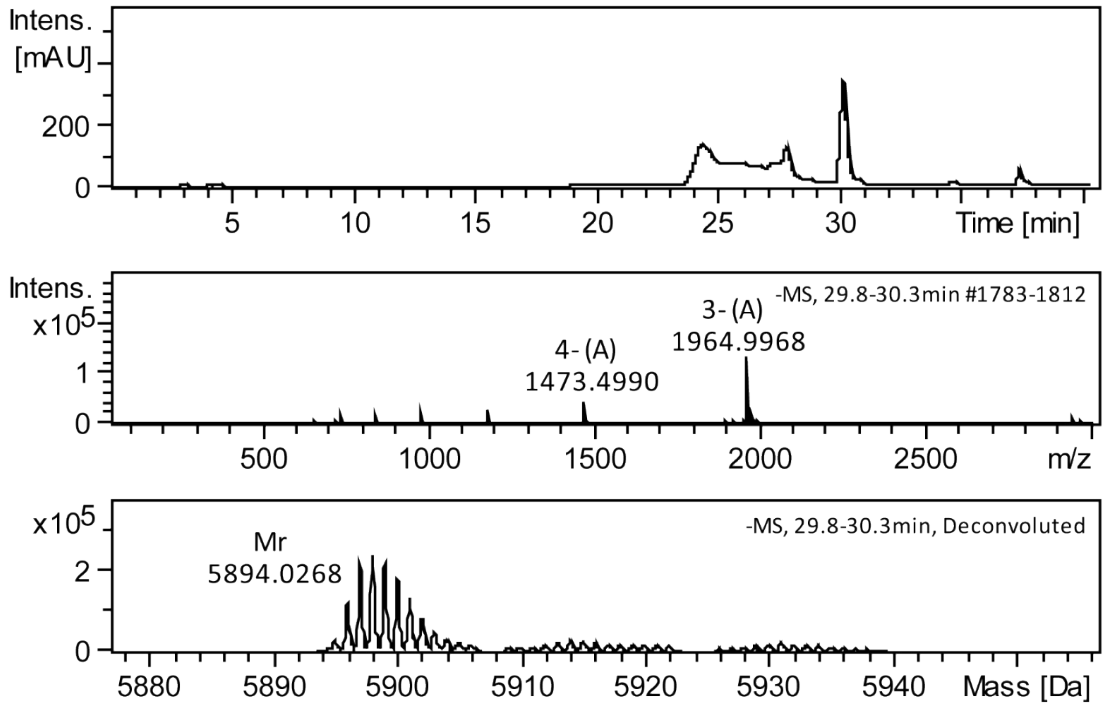


Figure S13. LC-MS chromatogram of the irradiated oligonucleotide **1A-irr** (crude).

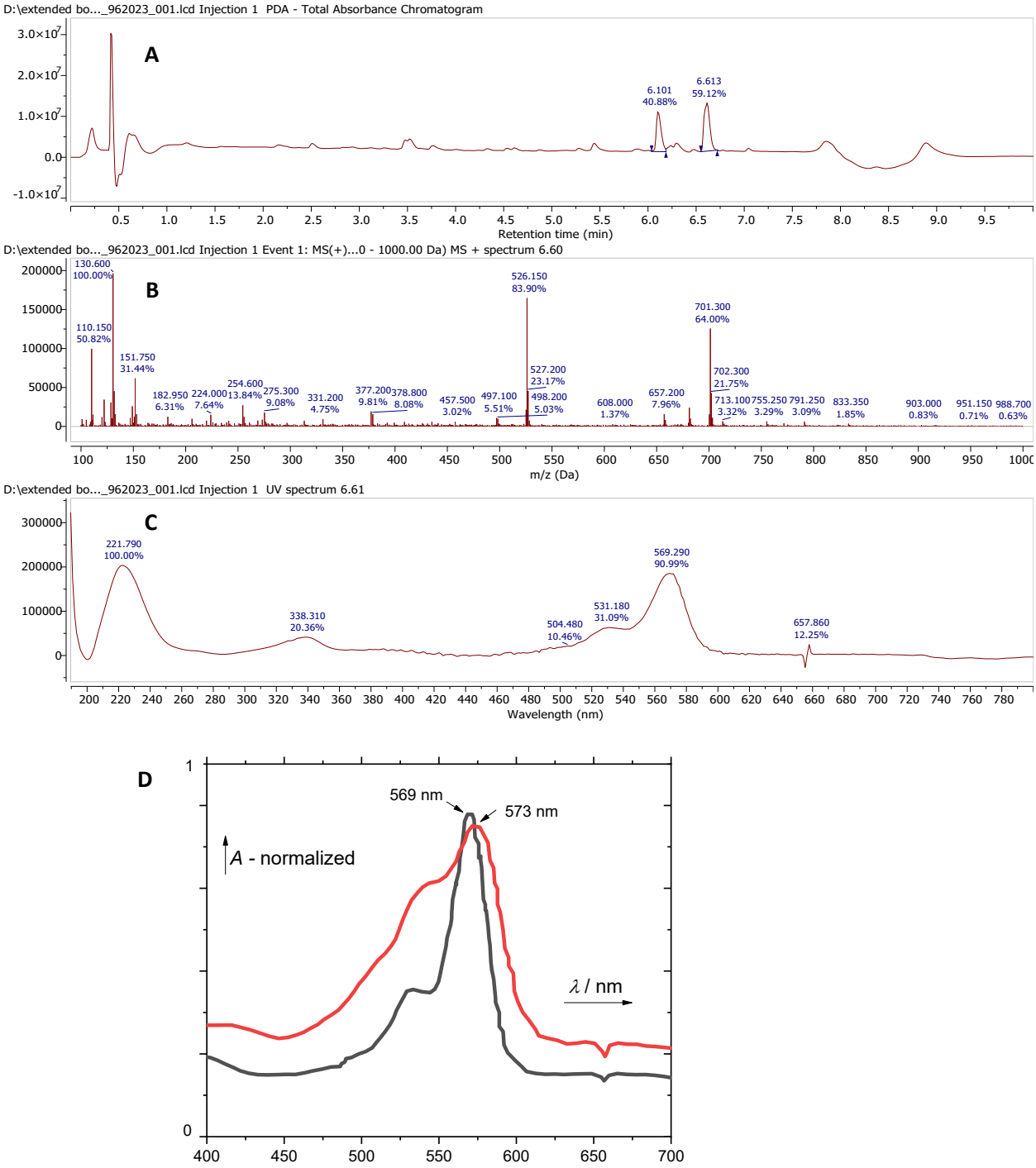


Figure S14. LC-MS analysis of **1** irradiated in DMSO/water ($c \approx 6 \cdot 10^{-6}$ M) with 625-nm LED. A) Total absorbance chromatogram. The peak at r.t. = 6.1 min corresponds to the fully photolyzed phototether **1-irr**. The peak at r.t. = 6.6 min corresponds to the oxidation product of the dioxetane intermediate **1-diox**, presumably to a vicinal diketone. B) extracted mass spectrum from the peak at r.t. = 6.6 min. m/z = 701. C) UV-vis spectrum extracted from the peak at r.t. = 6.6 min. D) overlap of normalized absorption spectra of peaks at r.t. = 6.1 min (**1-irr**, red) and at r.t. = 6.6 min (oxidation product of the dioxetane intermediate **1-diox**, presumably a vicinal diketone, black).

^{1}H NMR, ^{13}C NMR, COSY and HRMS spectra

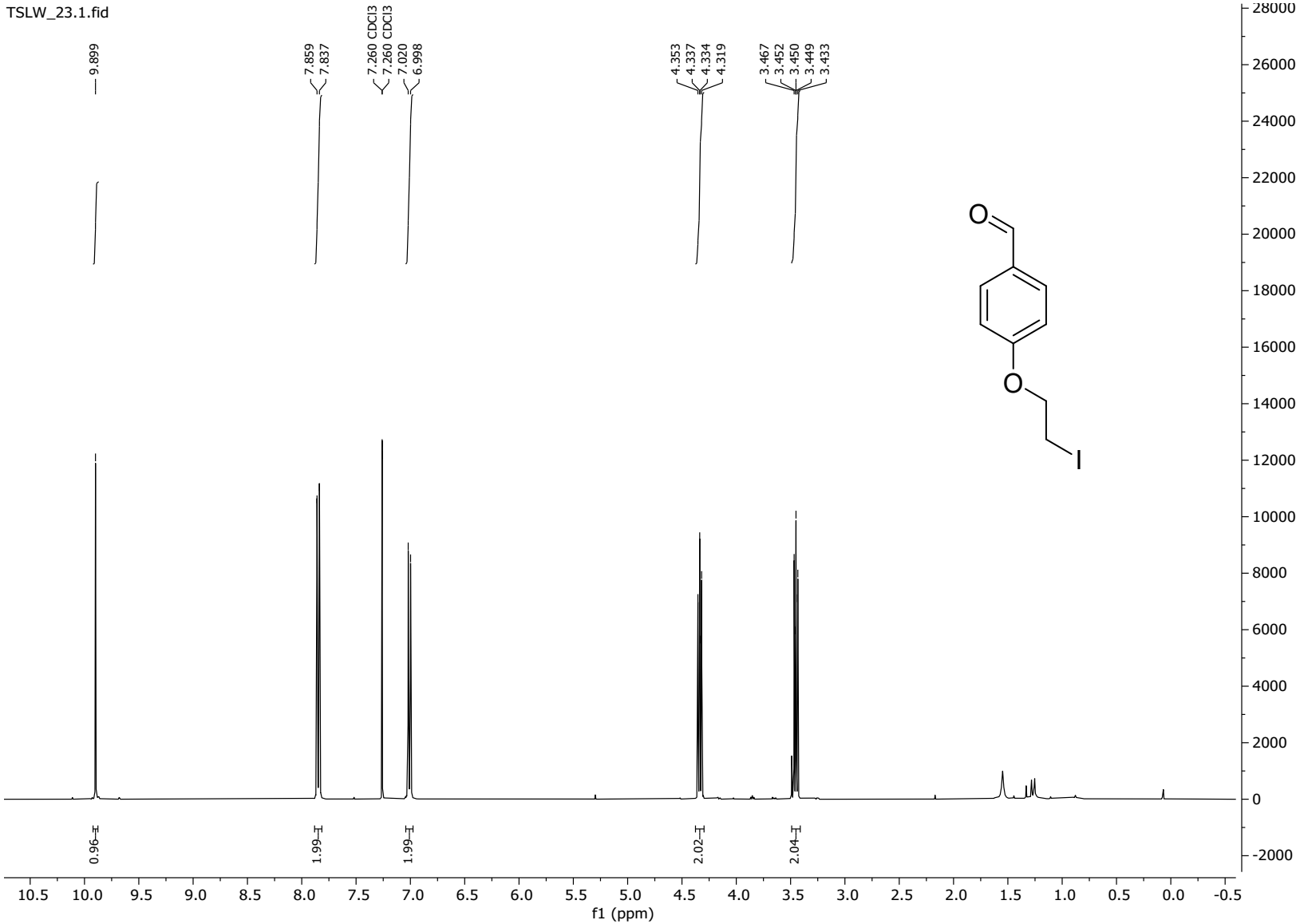


Figure S15. ^{1}H NMR of **3** in $CDCl_3$ (400.13 MHz)

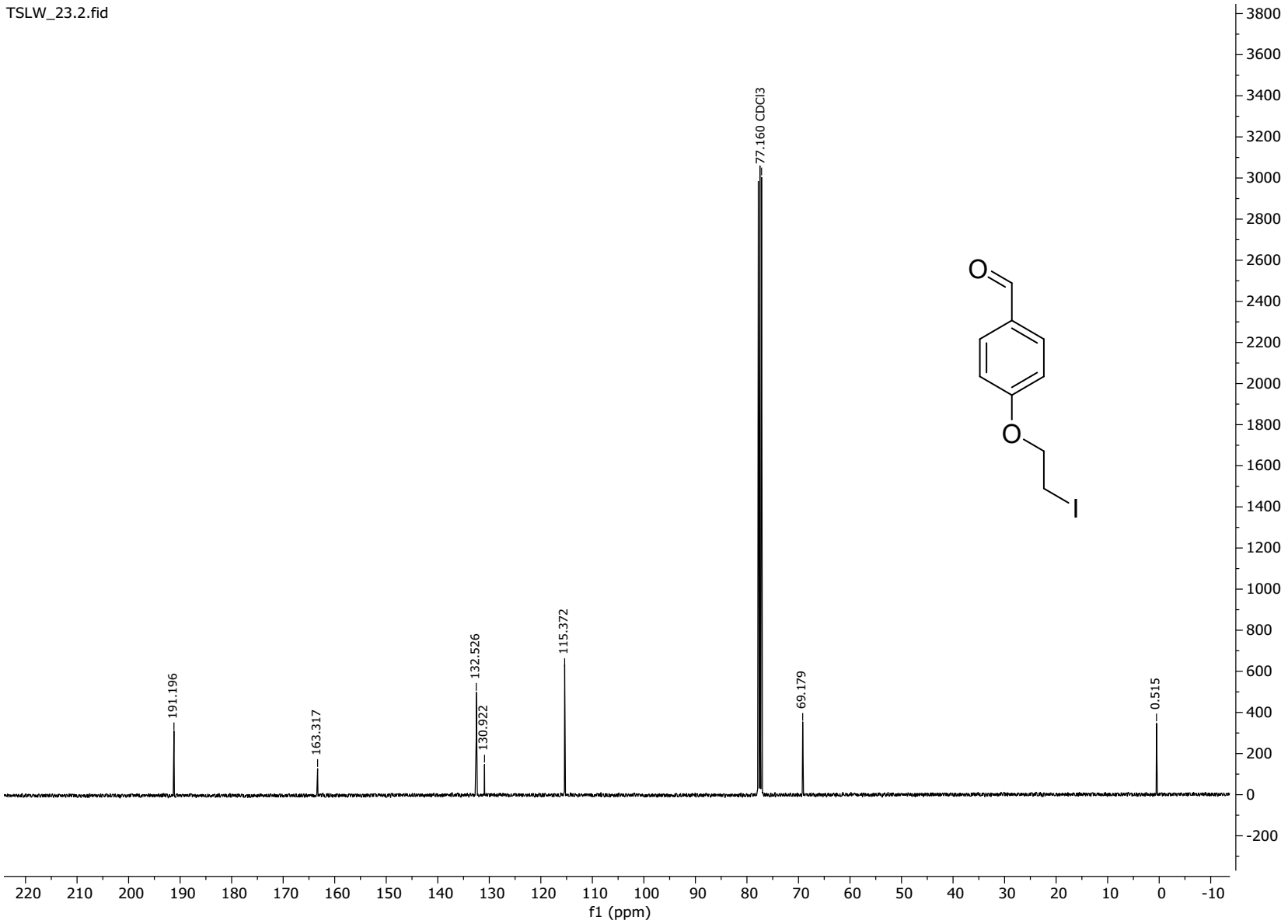
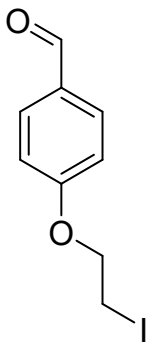


Figure S16. ^{13}C NMR of **3** in $CDCl_3$ (100.62 MHz).

Elemental Composition Report **Page 1**

Single Mass Analysis
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0
Element prediction: Off

Monoisotopic Mass, Odd and Even Electron Ions
5 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-9 H: 0-9 O: 0-2 I: 0-1



Wohrablova, TSLW_26 1071 (11.984) Cm (1071-(777:834+780:803))

TOF MS EI+
5.77e+003

Minimum:				-1.5		
Maximum:		5.0	5.0	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
275.9646	275.9647	-0.1	-0.4	5.0	0.8	C9 H9 O2 I

Figure S17. HR-MS analysis of **3**.

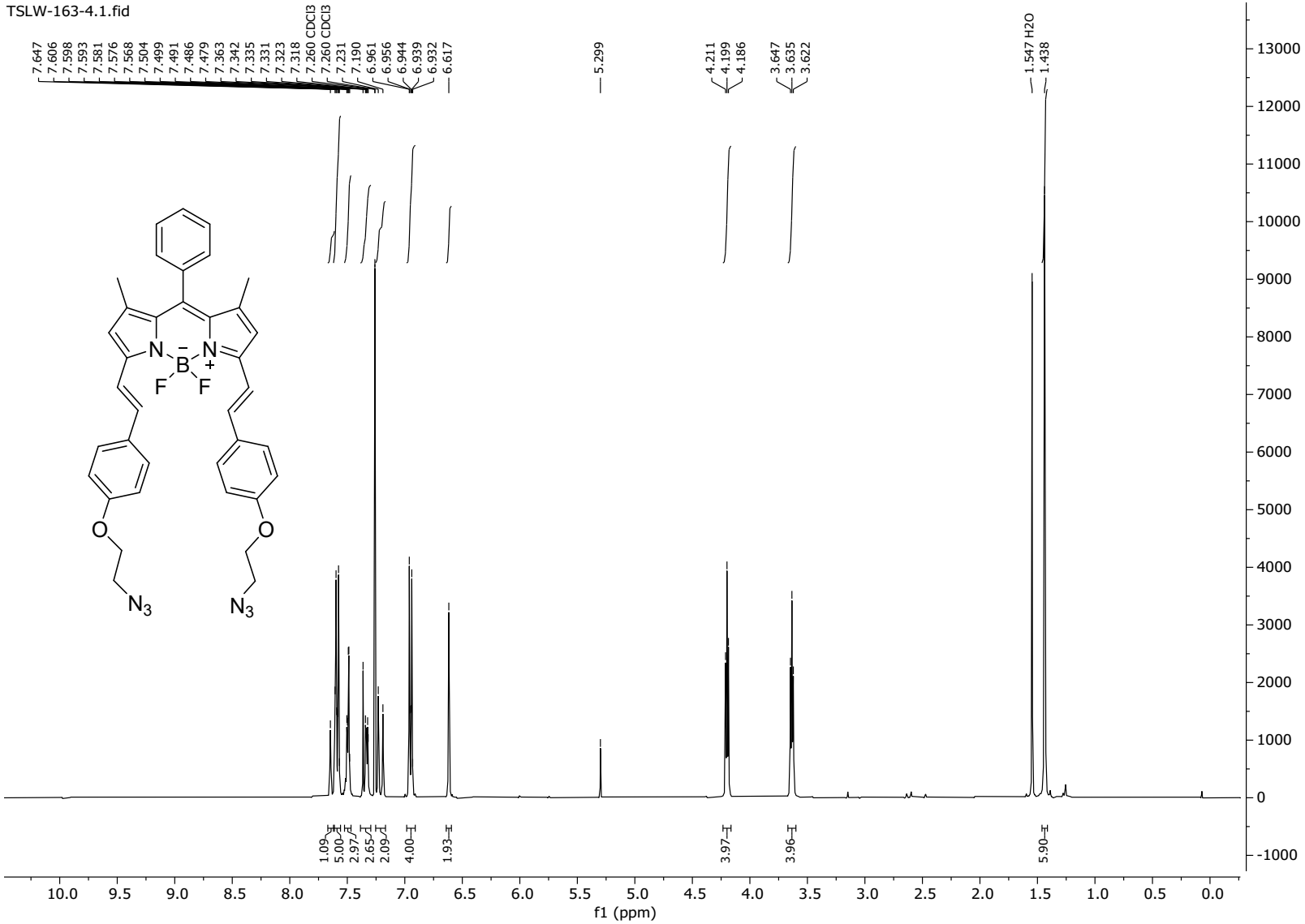


Figure S18. ^{1}H NMR **of 1** in $CDCl_3$ (400.13 MHz).

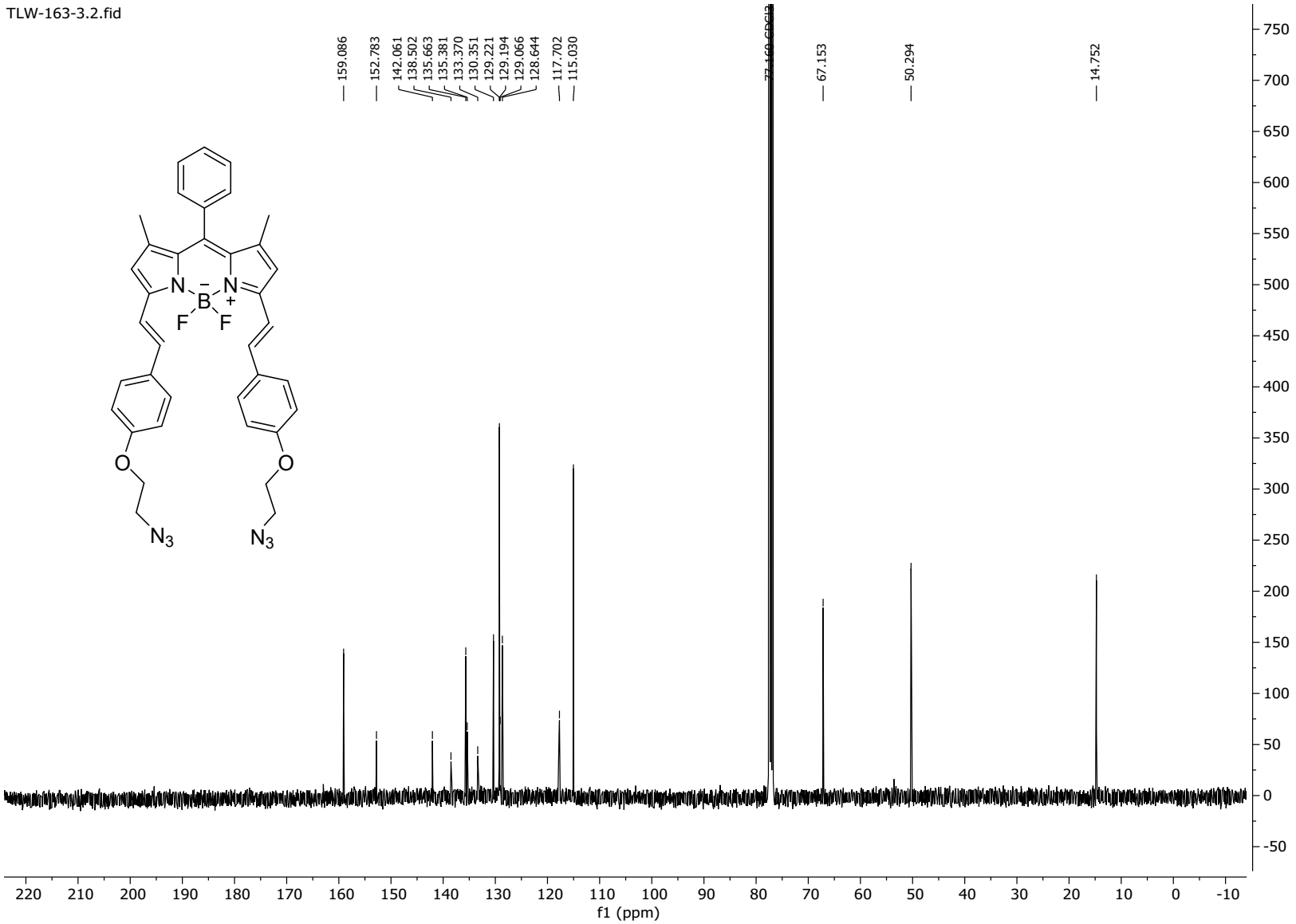


Figure S19. ^{13}C NMR of **1** in $CDCl_3$ (100.62 MHz).

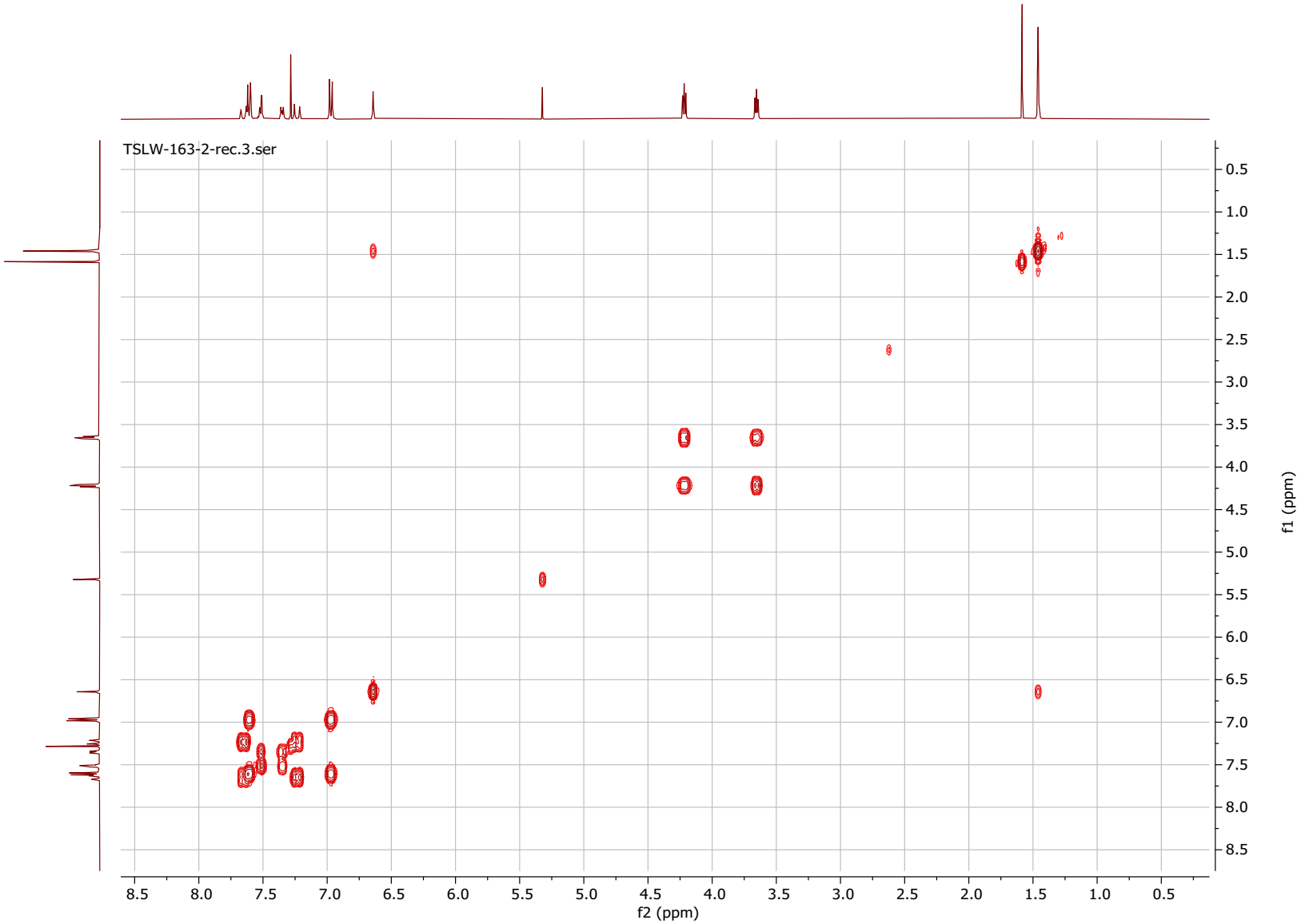


Figure S20. COSY of **1** in $CDCl_3$ (400.13 MHz).

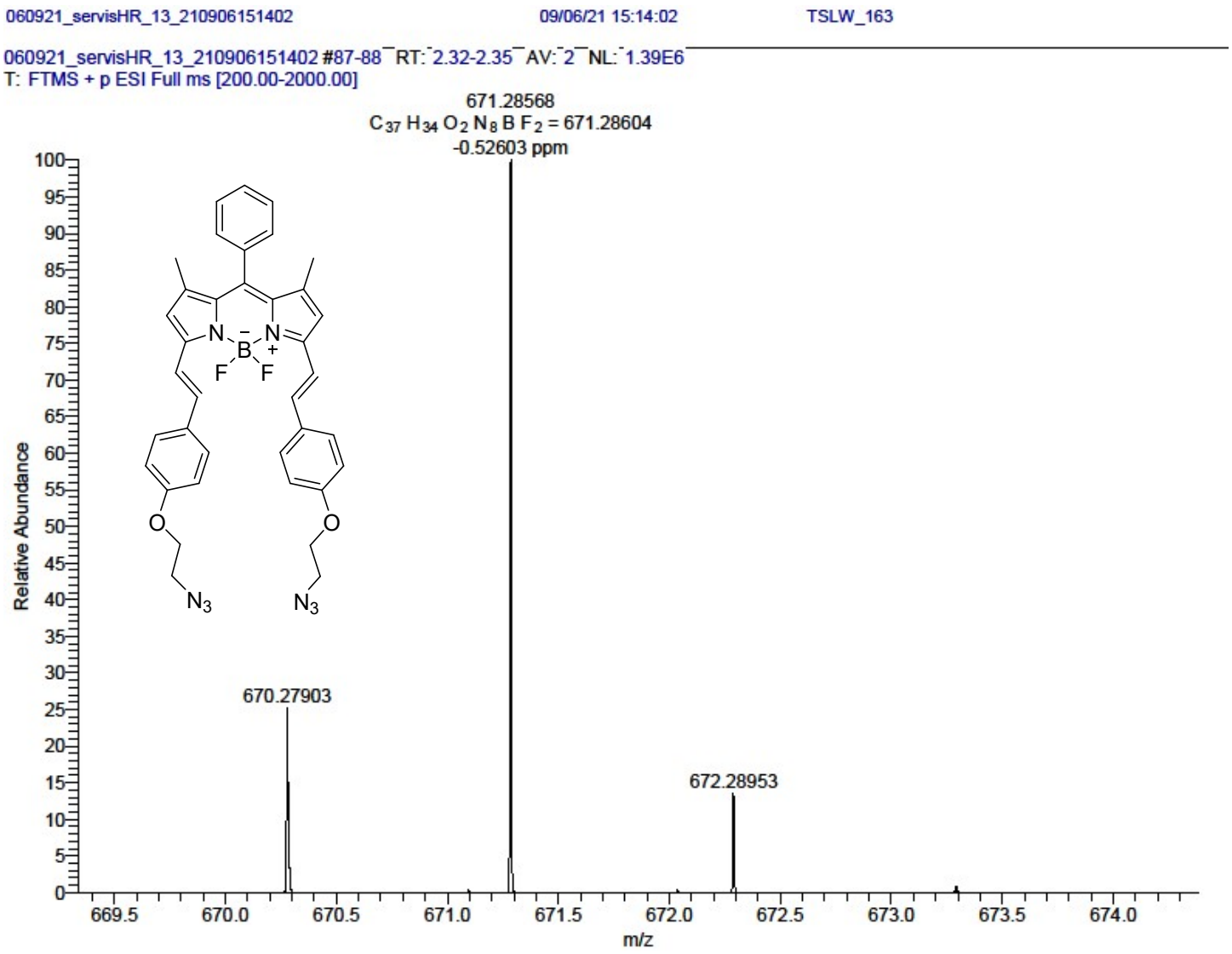


Figure S21. HR-MS analysis of **1**.

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