Supporting Information

Red-Emissive Guanylated Polyene-Functionalized Carbon Dots Arm Oral Epithelia against Invasive Fungal Infections

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TABLE OF CONTENT

TABLE OF CONTENT 2

MATERIALS AND METHODS 3

SYNTHESIS 3

CHARACTERIZATION 5

QUANTIFICATION OF AMB ON CD-GU^{+}-AMB 6

BIOLOGICAL ASSAYS 7

NMR AND MASS SPECTRA 12

MATERIALS AND METHODS

Materials. 4-Formylbenzoic acid, *N*-hydroxysuccinimide (NHS), 2,4-dimethylpyrrole, 2,3-Dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) and boron trifluoride ethyl etherate ($BF_3{\cdot}OEt_2$) were purchased from J&K chemical company and used as received. 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) was purchased from Meryer, and ethylenediamine and triethylamine (NEt_3) were ordered from Dieckmann.

NMR spectroscopy. The ^1H NMR and $^{13}C\{^1H\}$ NMR were obtained from Bruker Advance–III 400 spectrometers operating at 101 and 400 MHz, accordingly, and the chemical shifts are quoted in ppm. ^1H and ^{13}C chemical shifts were assigned relatively to solvent chemical shift values ($CDCl_3$: ^1H, 7.26 ppm; ^{13}C, 77.16 ppm; CD_3OD: ^1H, 3.31 ppm; ^{13}C, 49.00 ppm; $(CD_3)_2CO$: ^1H, 29.84 ppm; ^{13}C, 206.26 ppm), and the data were processed by MestReNova Software (Mestrelab).

Mass Spectroscopy. Bruker Autoflex Mass Spectrometer (MALDI–TOF) and Thermo Fisher Scientific UPLC–Q exactive focus hybrid quadrupole-orbitrap mass spectrometer in positive ion mode (ESI–MS) were performed to gather the high-resolution mass spectra.

SYNTHESIS

General scheme

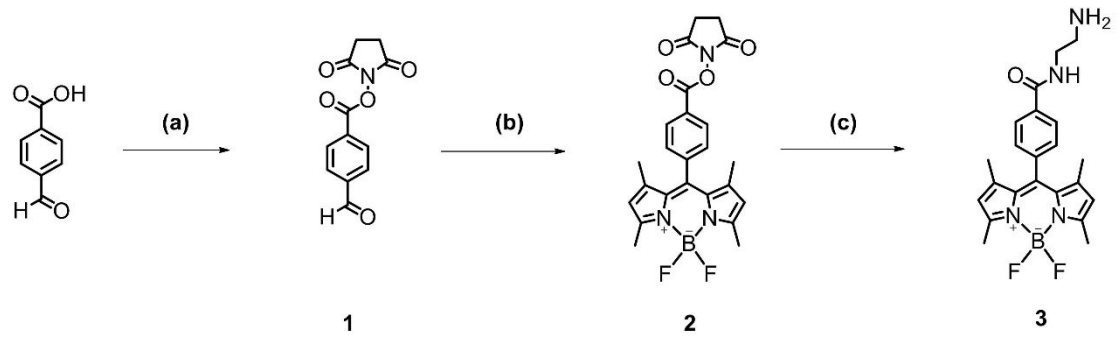

Figure S1 Synthetic scheme of BODIPY dye (**Compound 3**). (a) EDC, NHS, rt, 24 h; (b) (i) 2,4-dimethylpyrrole, TFA, CH_2Cl_2, rt, 24 h; (ii) DDQ, NEt_3, $BF_3{\cdot}OEt_2$, rt, 24 h; (c) ethylenediamine, CH_2Cl_2, rt, 12 h.

Compound 1. 4-Formylbenzoic acid (1.0 g, 6.6 mmol), EDC·HCl (2.0 g, 10.4 mmol) and NHS (2.2 g, 19.1 mmol) were dissolved in CH_2Cl_2 (25 mL). The mixture was stirred for 24 h at room temperature. The solvent was removed by rotary evaporator and the residue was re-dissolved in EtOAc (50 mL). The organic layer was washed with water (3 × 25 mL) and then brine (25 mL). The organic layer was dried with anhydrous $MgSO_4$. The product was then purified by flash silica gel column chromatography using EtOAc/n-hexane (1:1 = *v/v*) as eluent to afford **compound 1** as white solid. Yield: 1.02 g, 62%. ^{1}H NMR (400 MHz, $CDCl_3$, 298 K) δ 10.14 (s, 1H), 8.31 (d, *J* = 8.5 Hz, 2H), 8.03 (d, *J* = 8.6 Hz, 2H), 2.94 (s, 4H) (Figure S8). ^{13}C NMR (101 MHz, $CDCl_3$, 298 K) δ 191.30, 169.06, 161.19, 140.47, 131.34, 130.13, 129.87, 25.83 (Figure S9).

Compound 2. Compound 1 (0.71 g, 2.87 mmol) and 2,4-dimethylpyrrole (0.56 g, 5.88 mmol) were dissolved in degassed CH_2Cl_2 (450 mL). Ten drops of trifluoroacetic acid were added slowly, and the mixture was allowed to stir at room temperature for 24 h. DDQ (0.75 g, 3.3 mmol) was added to the mixture and allowed to react for 4 h at room temperature. Then triethylamine (NEt_3) (4.8 mL) was added followed by $BF_3{\cdot}OEt_2$ (4.8 mL). The mixture was further stirred for 24 h at room temperature. The organic layer was washed with water (3 × 300 mL). The organic layer was concentrated, and the product was purified by silica gel column chromatography using EtOAc/n-hexane (1:1 = *v/v*) as eluent to afford **Compound 2** as orange solid. Yield: 0.26 g, 19.5%. ^{1}H NMR (400 MHz, $CDCl_3$, 298 K) δ 8.27 (d, *J* = 8.3 Hz, 2H), 7.50 (d, *J* = 8.3 Hz, 2H), 6.00 (s, 2H), 2.93 (s, 4H), 2.56 (s, 6H), 1.38 (s, 6H) (Figure S10). ^{13}C NMR (101 MHz, $CDCl_3$, 298 K) δ 169.35, 161.44, 156.50, 142.99, 142.23, 139.39, 131.45, 130.86, 129.21, 125.93, 121.83, 25.86, 14.96, 14.79 (Figure S11). HRMS calculated for $C_{24}H_{22}N_3O_4BF_2$ $[M]^+$: 465.1670, found: 465.1658 (Figure S12).

Compound 3. Compound 2 (48.3 mg, 0.10 mmol) in CH_2Cl_2 (10 mL) was added dropwise to a CH_2Cl_2 solution (14 mL) containing 4 mL of ethylenediamine. The mixture was stirred for

12 h. The mixture was washed with water (30 mL) and then brine (30 mL). The organic layer was collected and dried with anhydrous Na_2SO_4. The product was then purified by silica gel chromatography using CH_2Cl_2/MeOH (10:1 = *v*/*v*) as eluent to afford **compound 3** as orange solid. Yield: 42 mg, 98.6%. ^{1}H NMR (400 MHz, $CDCl_3$, 298 K) δ 7.96 (d, *J* = 8.3 Hz, 2H), 7.38 (d, *J* = 8.3 Hz, 2H), 5.98 (s, 2H), 3.55 (q, *J* = 5.7 Hz, 2H), 3.00 (t, *J* = 5.7 Hz, 2H), 2.55 (s, 6H), 1.35 (s, 6H), –NH_2 proton signal missing (Figure S13). ^{13}C NMR (101 MHz, $CDCl_3$, 298 K) δ 166.83, 156.04, 143.07, 140.46, 138.44, 135.17, 131.18, 128.58, 127.98, 121.58, 42.43, 41.28, 14.78, 14.75 (Figure S14). HRMS calculated for $C_{22}H_{25}BF_2N_4O$ $[M+H]^+$: 410.2088, found 410.2085 (Figure S15).

CHARACTERIZATION

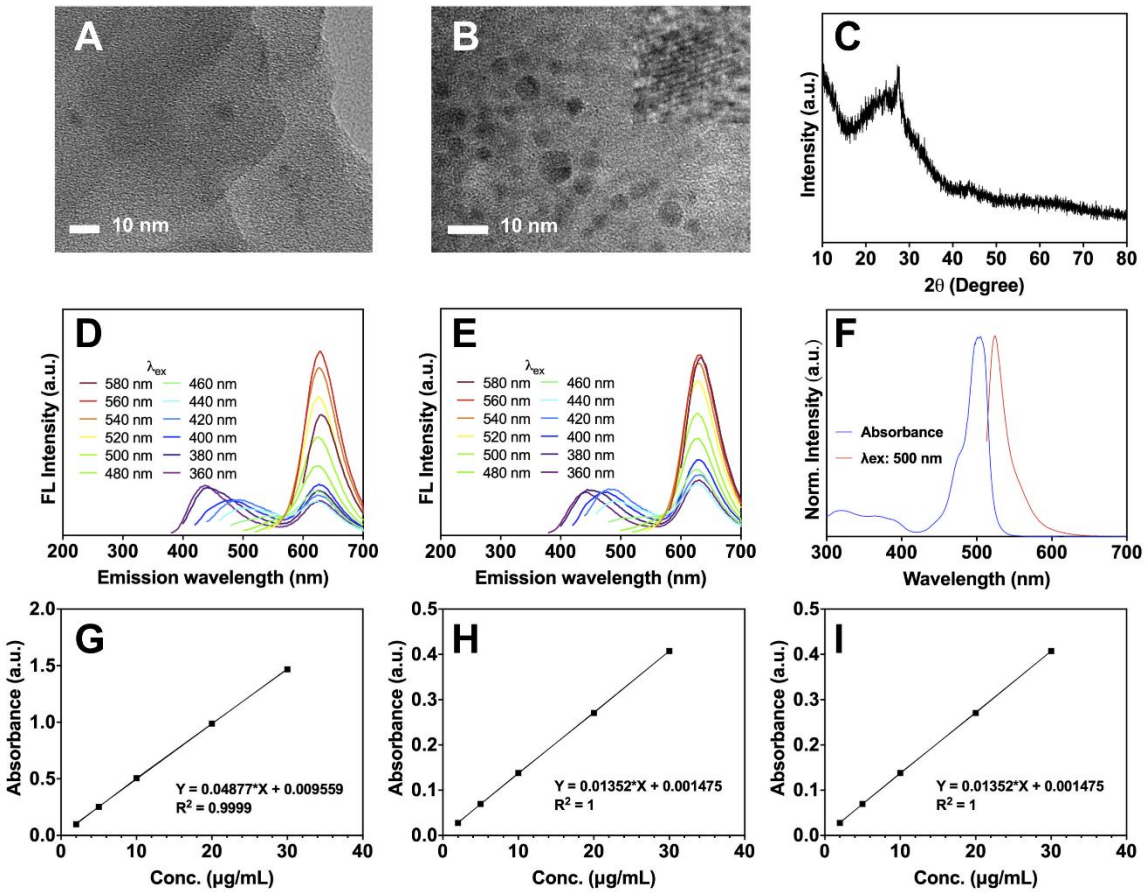

Figure S2 TEM images of (A) CD-Gu^+ and (B) CD-Gu^+-AmB. (C) The XRD patterns with wide angle at approximately 25°, and FL spectra of (D) CD and (E) CD-Gu^+ exhibiting dual

emission peaks. (F) UV–vis and FL spectrum of NH_2-bodipy. The calibration curves of CD-Gu^+ at (G) 534, (H) 417 nm, and free AmB at (I) 417 nm.

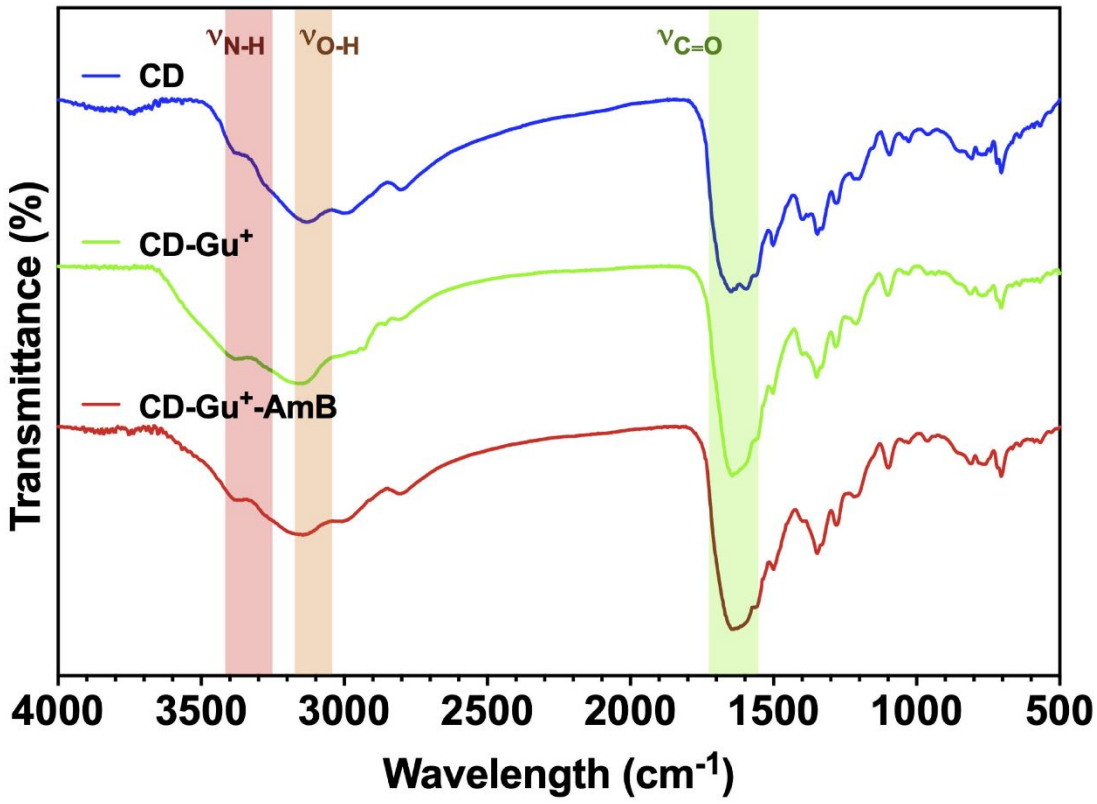

Figure S3 FT–IR of CD, CD-Gu^+ and CD-Gu^+-AmB.

QUANTIFICATION OF AMB ON CD-GU$^+$-AMB

To estimate the conjugated AmB quantity, the calibration curves of CD-Gu^+ and free AmB were first obtained by using the Beer's Law. After obtaining the equations, the absorbance of CD-Gu^+-AmB was matched with the absorbance of CD-Gu^+ at 535 nm to determine the concentration of CD-Gu^+ in CD-Gu^+-AmB and used to calculate the baseline of each distinctive peak of AmB afterwards. Then, the calculated AmB baseline was subtracted from the measured AmB peak of CD-Gu^+-AmB, and the concentration of conjugated AmB was computed by plugging the AmB absorbance into the free AmB equation. Finally, concentration of AmB was divided by the concentration of CD-Gu^+-AmB to determine the wt%.

BIOLOGICAL ASSAYS

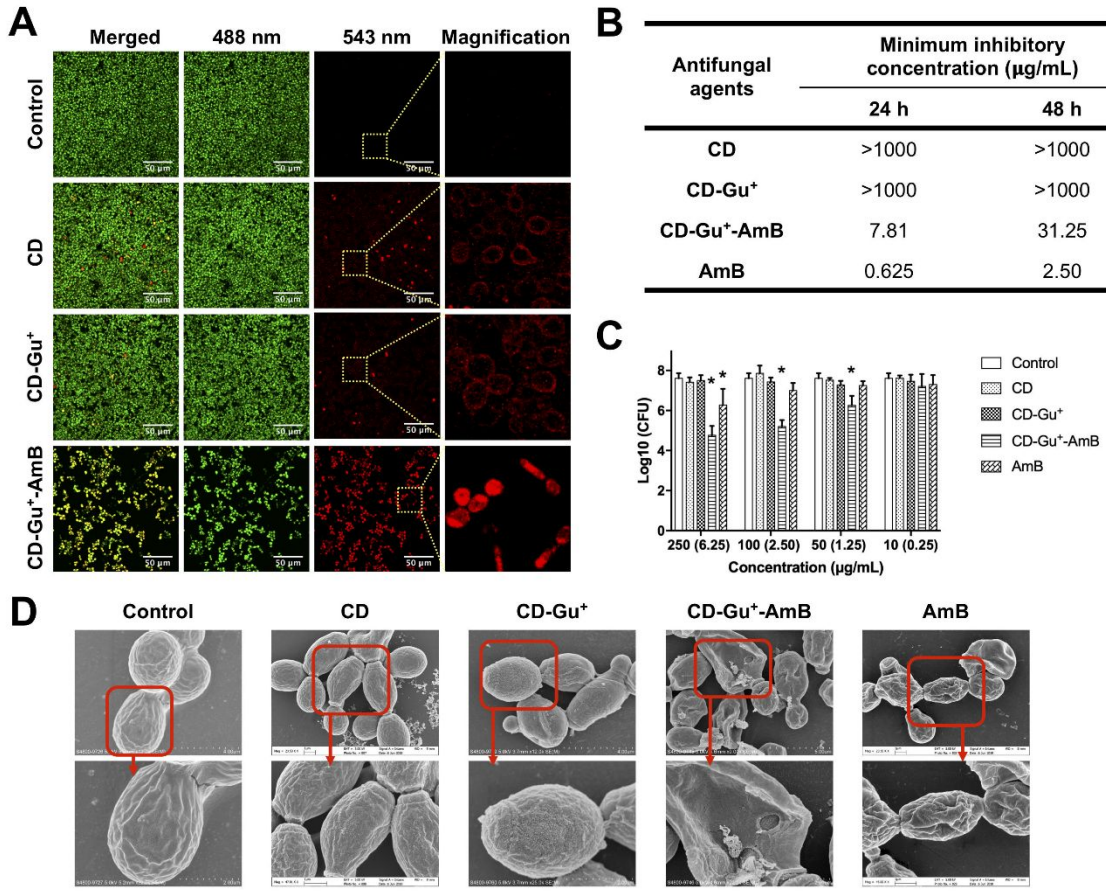

Figure S4 (A) Confocal images of CD, CD-Gu$^+$ and CD-Gu$^+$-AmB (100 μg/mL) treated two-day-old *C. albicans* (ATCC 90028) biofilms. The *Candida* cells were stained by SYTO9 (λ_{ex} = 488 nm) with green fluorescence, while the interacted CDs with red fluorescence were excited by 543 nm HeNe laser. (B) The minimal inhibitory concentration (MIC, μg/mL) of CD, CD-Gu$^+$, CD-Gu$^+$-AmB and AmB against the planktonic mode of *C. albicans* (ATCC 90028) at 24 and 48 h. (C) After 24 h treatment of CD, CD-Gu$^+$, CD-Gu$^+$-AmB with a series of concentrations (250, 100, 50 and 10 μg/mL) and AmB (6.25, 2.50, 1.25 and 0.25 μg/mL, equivalent to conjugated AmB of tested CD-Gu$^+$-AmB concentration), the concentrations of live *C. albicans* (ATCC 90028) in the two-day-old biofilms were presented as Log 10 of the colony-forming units (CFU). The assay was performed on three different occasions in duplicate, and the data are presented as mean ± SD. The asterisk indicates the significant

differences between the treatment and control groups ($p < 0.05$). (D) The morphology of *C. albicans* treated with CD, CD-Gu$^+$, CD-Gu$^+$-AmB (100 μg/mL) and AmB (2.50 μg/mL) were assessed using FEG–SEM.

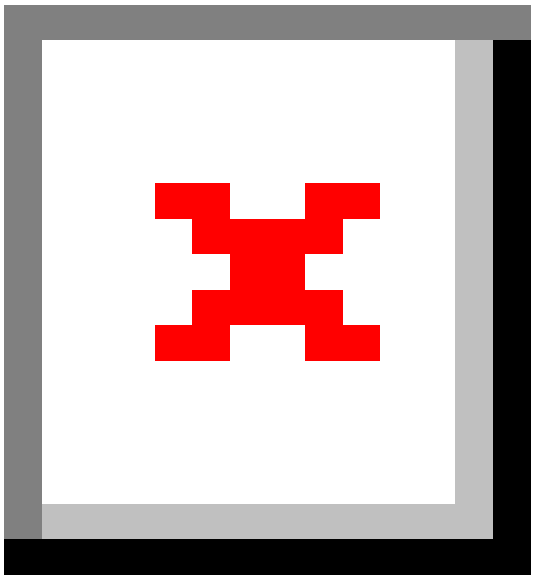

Figure S5 The fluorescent images of cryosections from RHOE treated with CD or CD-Gu$^+$ (200 μg/mL) for 0, 2, 6 and 12 h. The immunofluorescence staining of E-cadherin (green) was performed on the slices, and the cell nuclei were stained by DAPI (blue). The red channel represents CD/CD-Gu$^+$ in the tissue (A). After treating the tissues of CD/CD-Gu$^+$ (200 μg/mL) for 6 h, the RHOE were sequentially cultured in SkinEthic growth medium up to 8 d. The

tissues were collected at 6 h, 2, 4 and 8 d for the cryosection followed by immunofluorescence staining of E-cadherin (green), and the cell nuclei were stained by DAPI (blue). The images show the shifting of CD/CD-Gu^+ (red) in RHOE in the experimental period (B). (Scale bar: 50 μm)

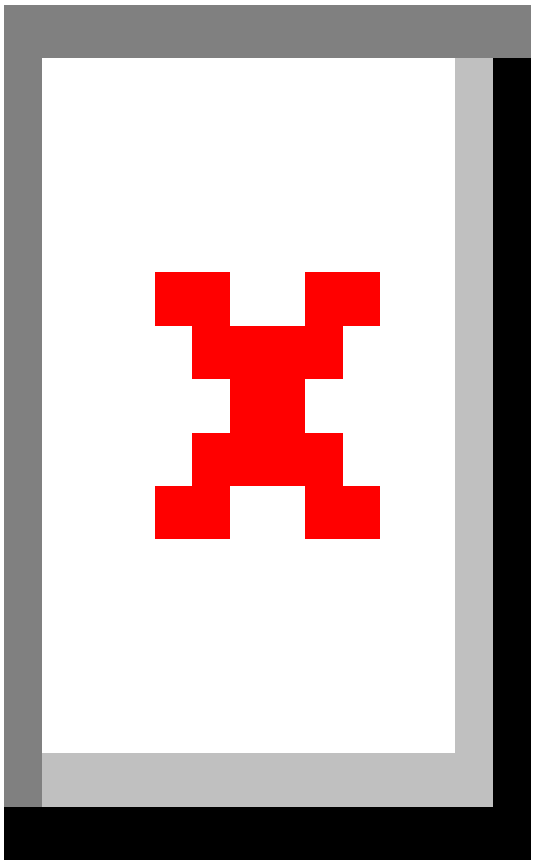

Figure S6 The fluorescent images of cryosections from reconstituted human gingival epithelia (RHGE) treated with CD, CD-Gu^{+} and CD-Gu^{+}-AmB (200 μg/mL) for 0, 2, 6 and 12 h. The

immunofluorescence staining of E-cadherin (green) was performed on the slices, and the cell nuclei were stained by DAPI (blue). The red channel represents CD/CD-Gu^+/CD-Gu^+-AmB in the tissue. (Scale bar: 50 μm)

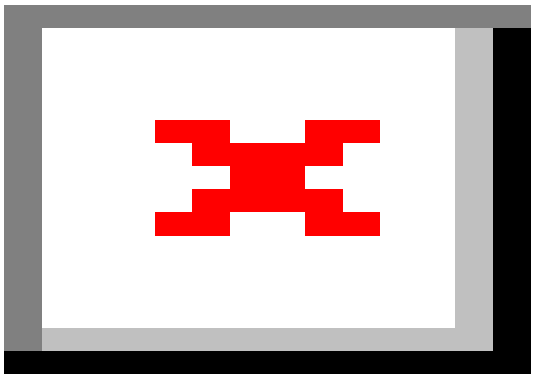

Figure S7 After pretreating RHOE with PBS, different CDs and AmB for 6 h, RHOE were subsequently challenged with *C. albicans* SC5314 (4×10^4 CFU/mL) for 24 and 48 h. The tissues at each time point were collected and cryosectioned for the fluorescent microscopy. The immunofluorescence staining of E-cadherin (green) was performed on the slices, and the cell nuclei were stained by DAPI (blue). The red channel represents different CDs in the tissue sections. (Scale bar: 50 μm)

NMR AND MASS SPECTRA

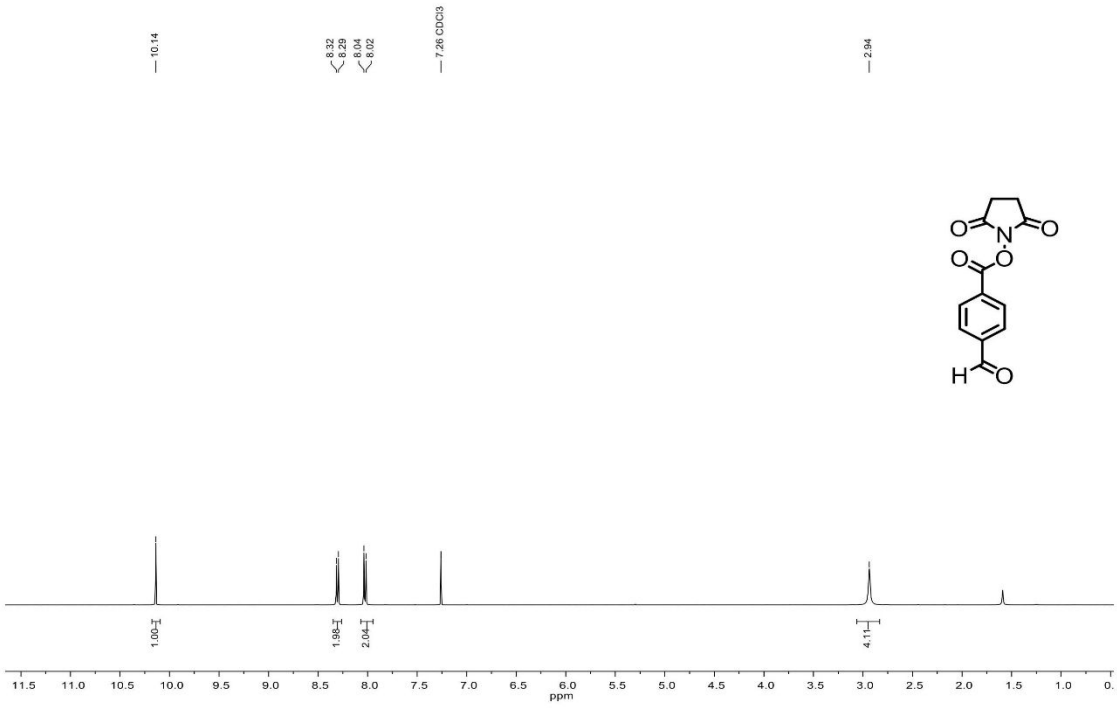

Figure S8 ^{1}H NMR spectrum (400 MHz, $CDCl_3$, 298 K) of **compound 1**.

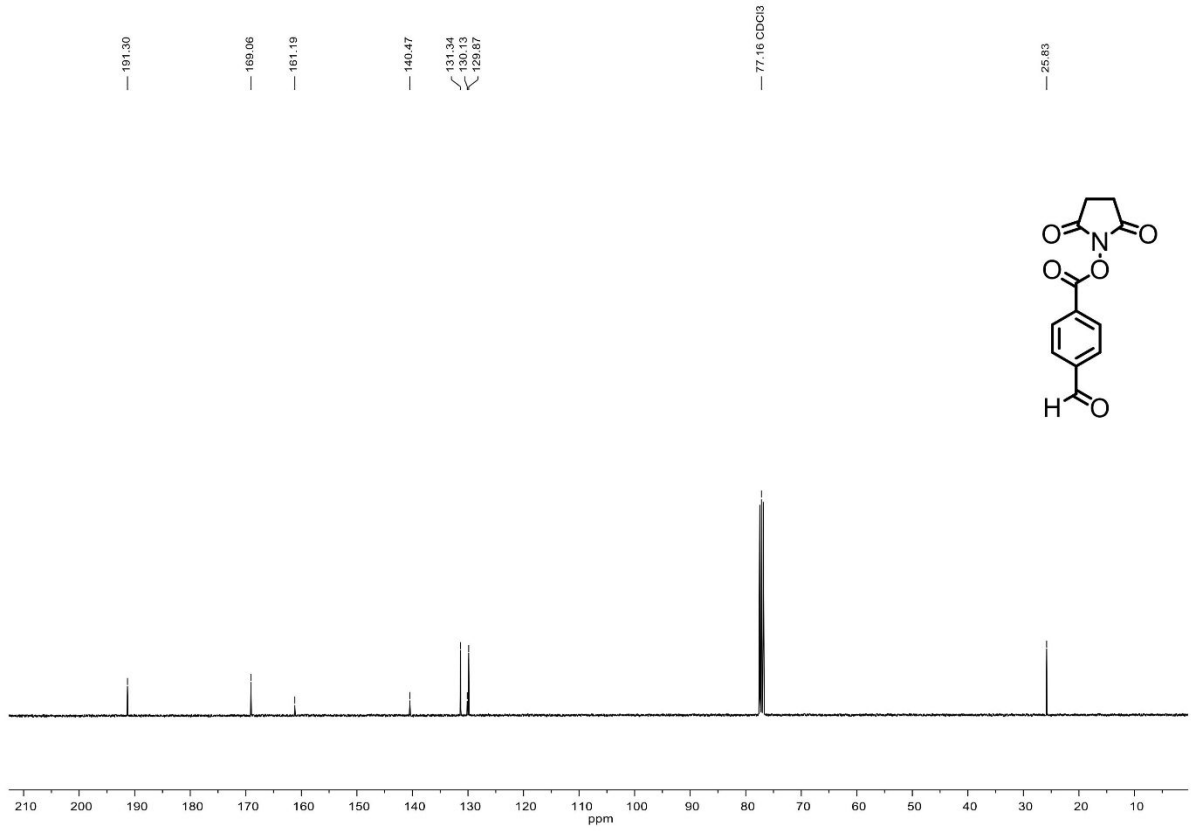

Figure S9 $^{13}C\{^{1}H\}$ NMR spectrum (101 MHz, $CDCl_3$, 298 K) of **compound 1.**

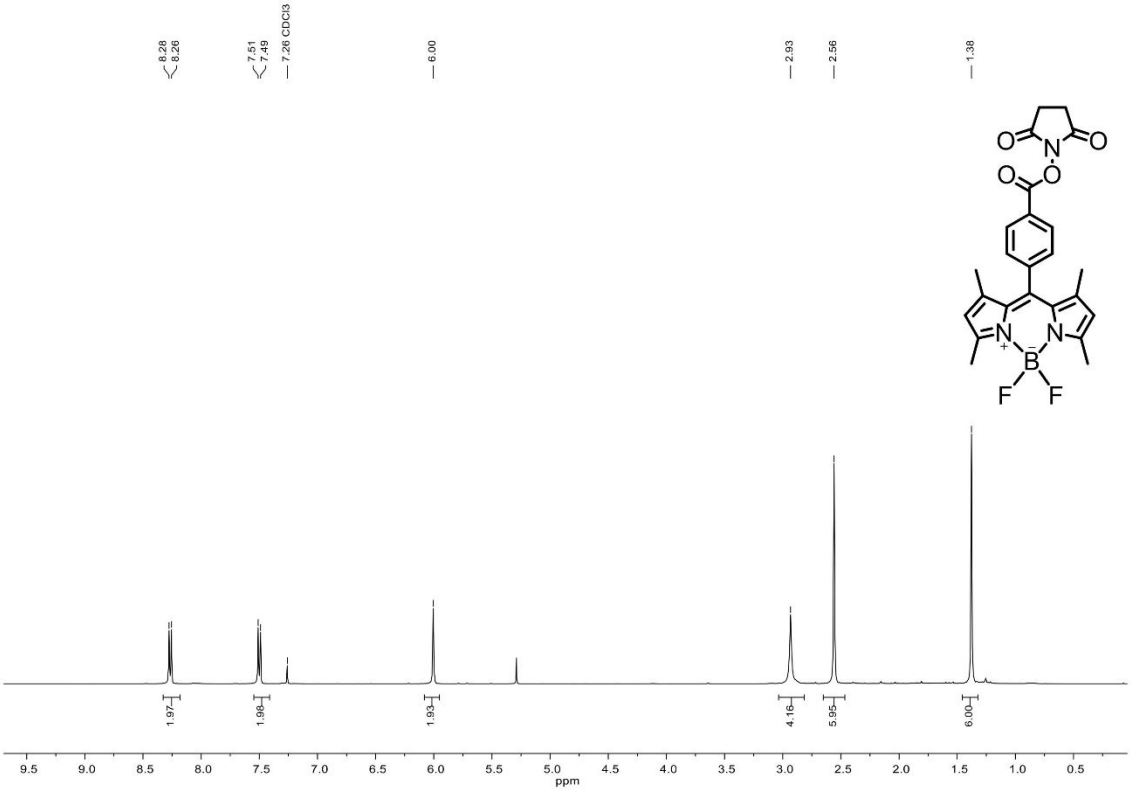

Figure S10 ^{1}H NMR spectrum (400 MHz, $CDCl_3$, 298 K) of **compound 2.**

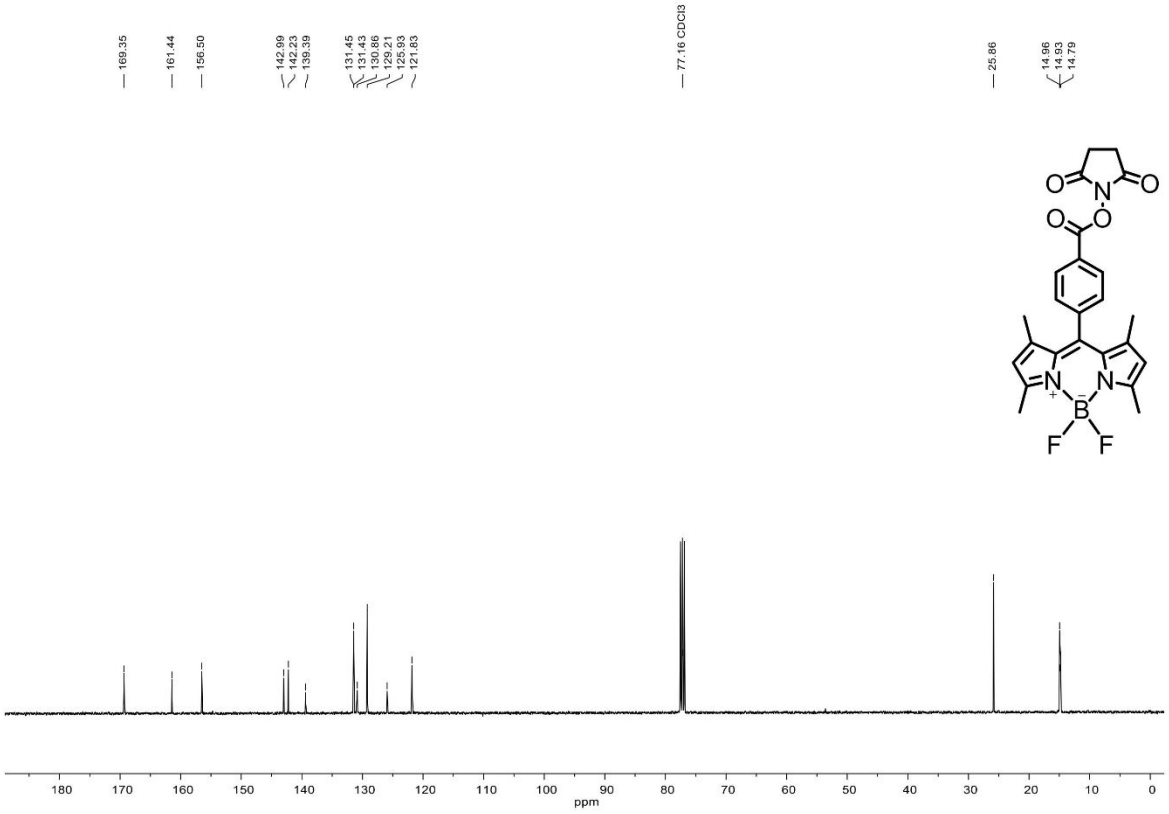

Figure S11 $^{13}C\{^{1}H\}$ NMR spectrum (101 MHz, $CDCl_3$, 298 K) of **compound 2**.

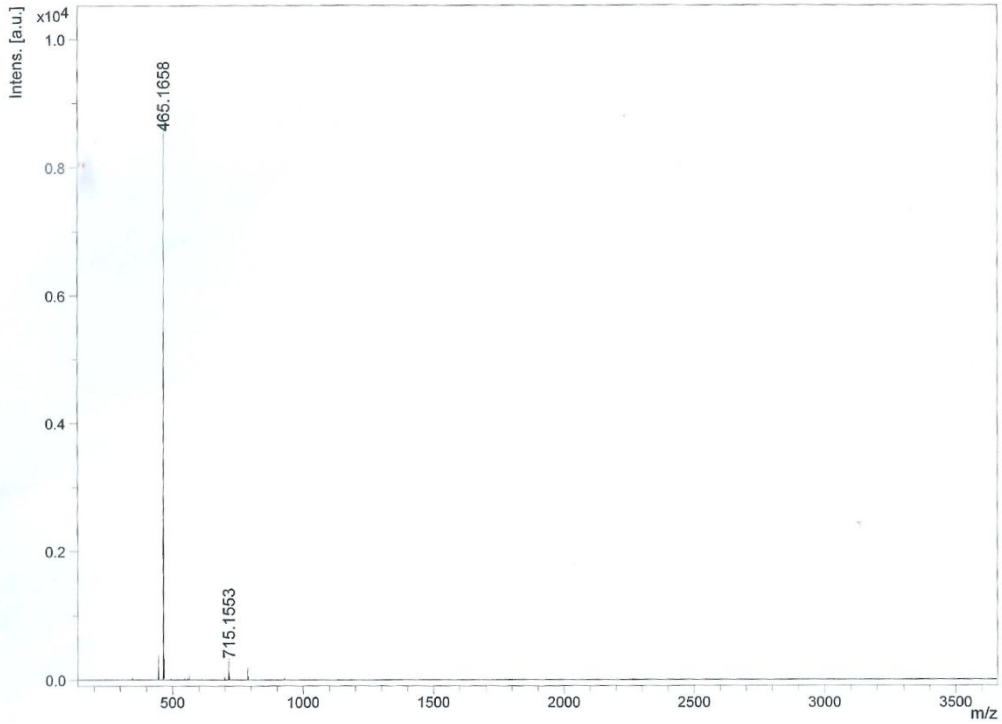

Figure S12 HRMS (MALDI–TOF) analysis of **compound 2**: calculated for $C_{24}H_{22}N_3O_4BF_2$ $[M]^+$ *m/z* 465.1670, found 465.1658.

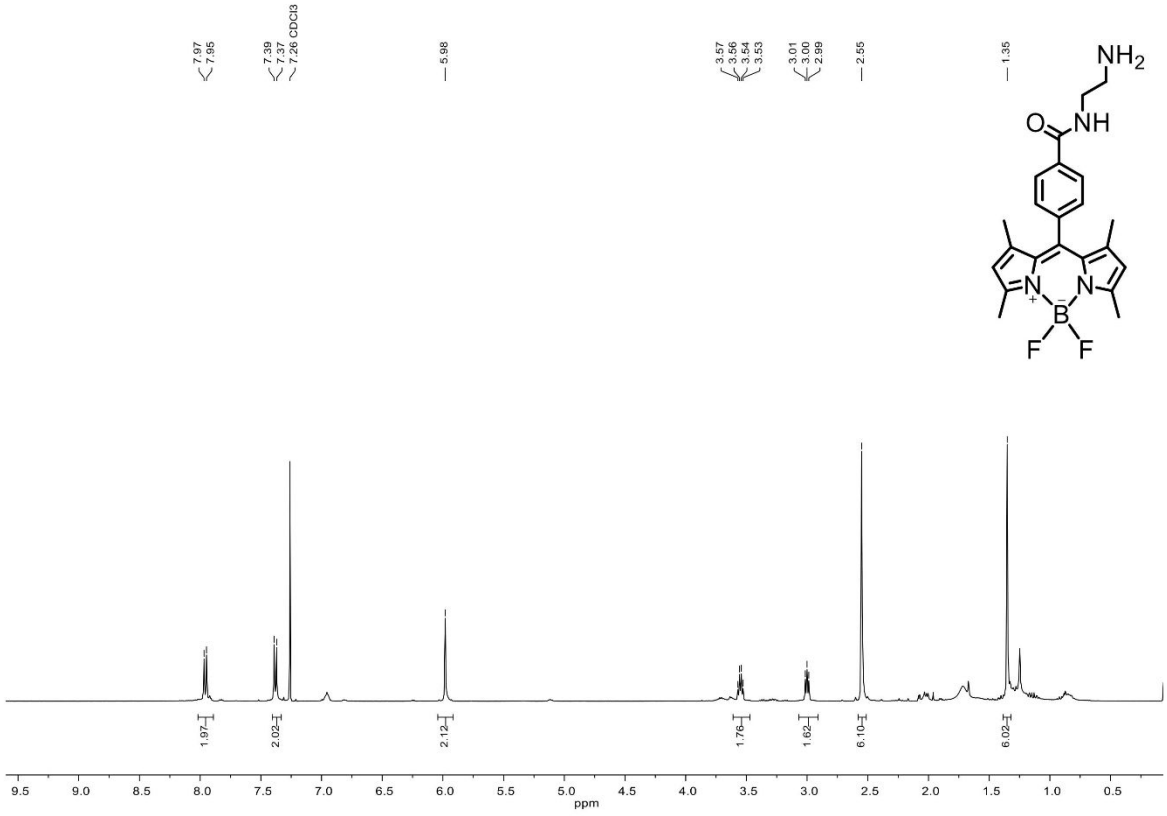

Figure S13 ^{1}H NMR spectrum (400 MHz, $CDCl_3$, 298 K) of **compound 3**.

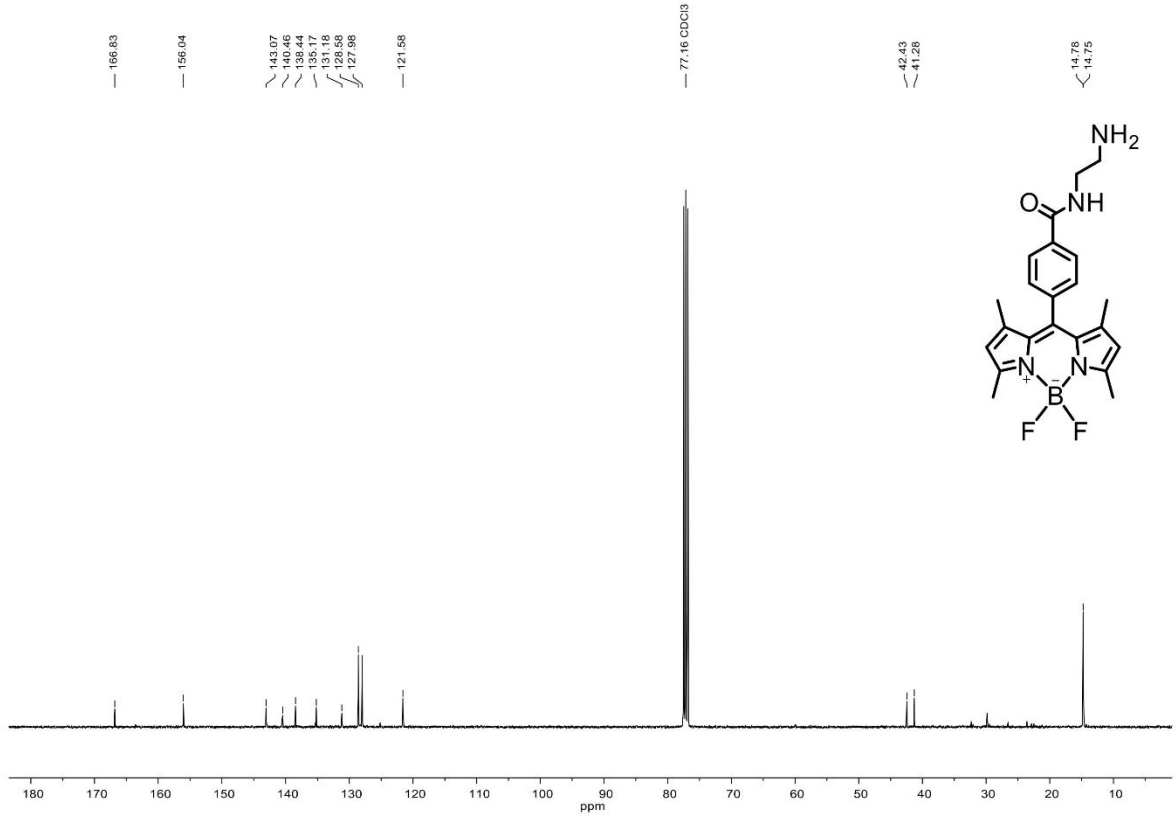

Figure S14 $^{13}C\{^{1}H\}$ NMR spectrum (101 MHz, $CDCl_3$, 298 K) of **compound 3**.

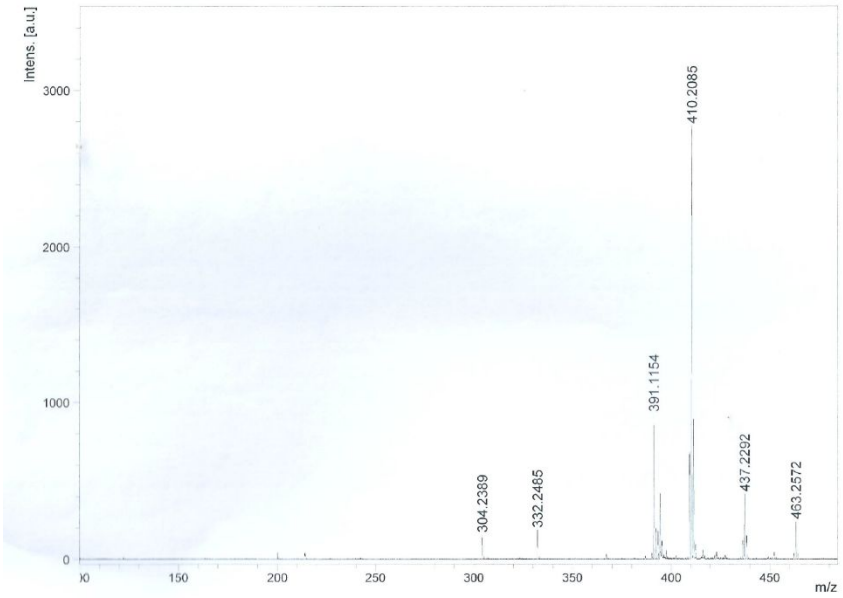

Figure S15 HRMS (MALDI–TOF) analysis of **compound 3**: calculated for $C_{22}H_{25}BF_2N_4O$ $[M+H]^+$ *m/z* 410.2088, found 410.2085.