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Supplementary Material

New boron-based coumarin fluorophores for bioimaging applications

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Confocal Microscopy

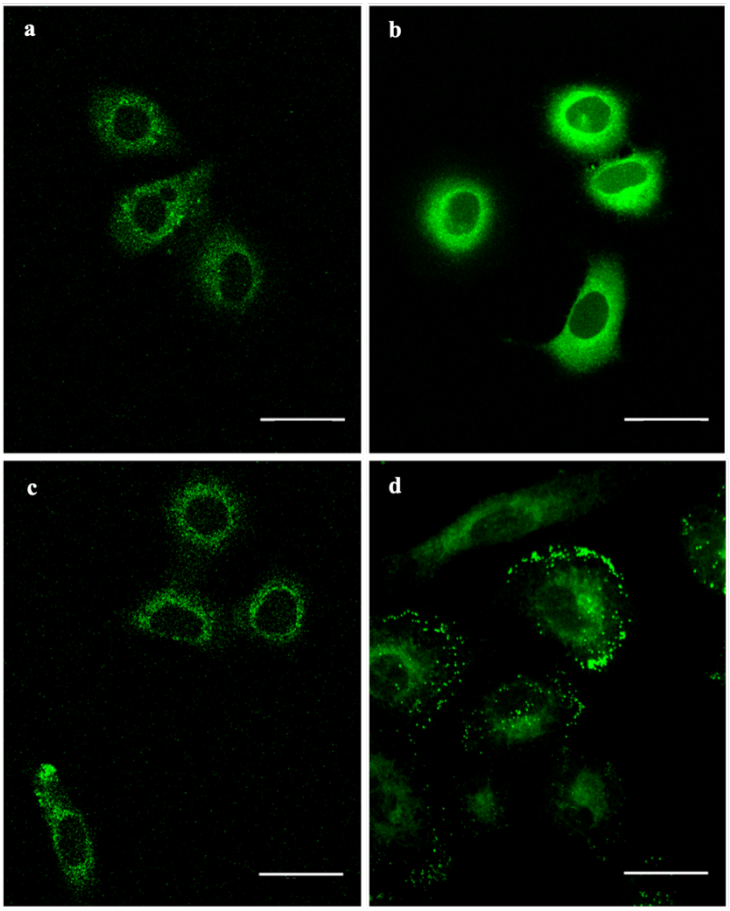

Figure S1: Confocal microscopy images of A549 cells treated with (a) **HCoBA** (10 μM, 20 min), (b) **HCpBA** (10 μM, 20 min), (c) **ICPh** (10 μM, 20 min), and (d) **ICCb** (10 μM, 20 min), showing coumarin fluorescence (λ_{ex} = 458 nm, λ_{em} = 468-568 nm). Scale bar represents 30 μm.

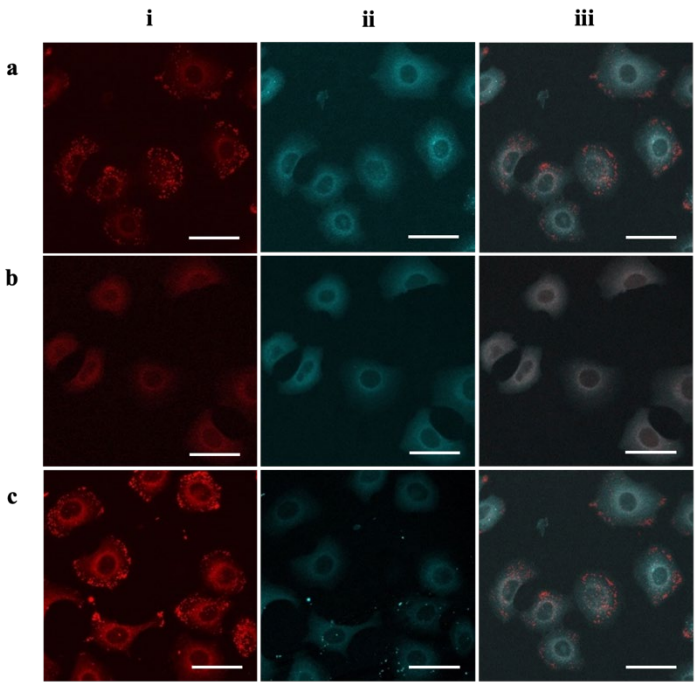

Figure S2. Confocal microscopy images of A549 cells treated with Nile Red (50 μM, 20 min) and (a) **HCoBA** (10 μM, 20 min), (b) **HCmBA** (10 μM, 20 min), or (c) **HCpBA** (10 μM, 20 min), showing (i) Nile Red fluorescence (λ_{ex} = 561 nm, λ_{em} = 571 - 700 nm), (ii) coumarin fluorescence (λ_{ex} = 458 nm, λ_{em} = 468 - 568 nm), and (iii) overlay of (i) and (ii). Pearson's correlation coefficient for treatment with Nile Red are: **HCoBA** (R = 0.27 ± 0.03), **HCmBA** (R = 0.34 ± 0.04), and **HCpBA** (R = 0.40 ± 0.03). Scale bar represents 30 μm.

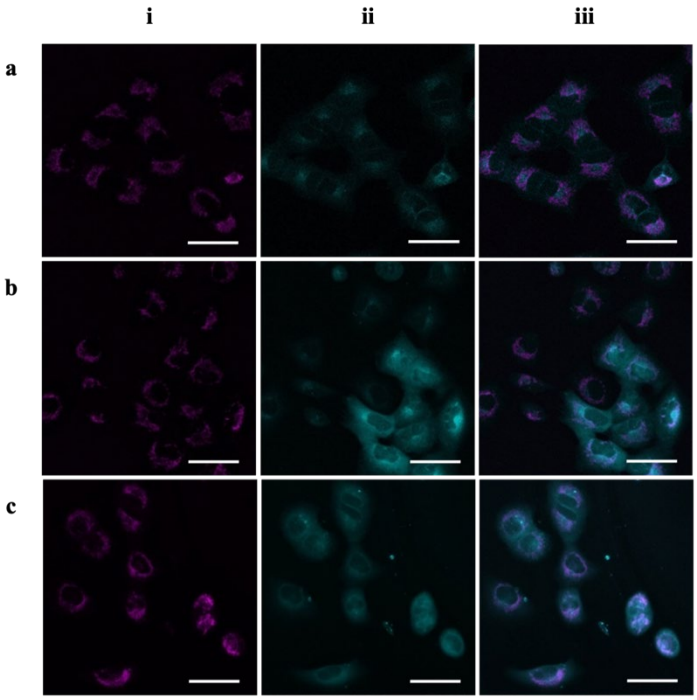

Figure S3. Confocal microscopy images of A549 cells treated with MitoTracker Red CMXRos (100 nM, 20 min) and (a) **HCoBA** (10 μM, 20 min), (b) **HCmBA** (10 μM, 20 min), or (c) **HCpBA** (10 μM, 20 min), showing (i) MitoTracker Red CMXRos fluorescence (λ_{ex} = 561 nm, λ_{em} = 570 - 767 nm), (ii) coumarin fluorescence (λ_{ex} = 458 nm, λ_{em} = 468 - 568 nm), and (iii) overlay of (i) and (ii). Pearson's correlation coefficient for treatment with MitoTracker Red CMXRos are: **HCoBA** (R = 0.22), **HCmBA** (R = 0.17), and **HCpBA** (R = 0.55). Scale bar represents 40 μm.

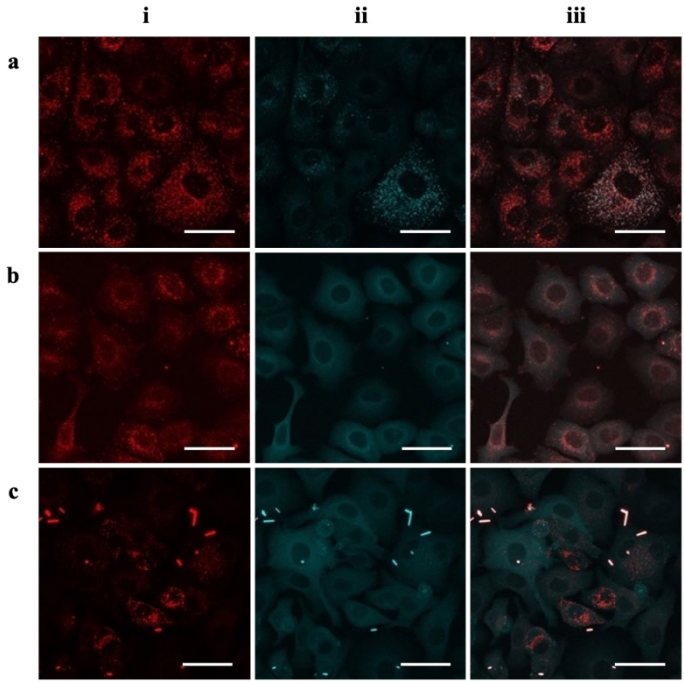

Figure S4. Confocal microscopy images of A549 cells treated with LysoTracker Red DND-99 (50 nM, 20 min) and (a) **HCoBA** (10 μM, 20 min), (b) **HCmBA** (10 μM, 20 min), or (c) **HCpBA** (10 μM, 20 min), showing (i) LysoTracker Red DND-99 fluorescence (λ_{ex} = 561 nm, λ_{em} = 568 - 701 nm), (ii) coumarin fluorescence (λ_{ex} = 458 nm, λ_{em} = 468 - 568 nm), and (iii) overlay of (i) and (ii). NB: Row c shows some evidence of probe precipitation. Pearson's correlation coefficient for treatment with LysoTracker Red DND-99 are: **HCoBA** (R = 0.30 ± 0.06), **HCmBA** (R = 0.33 ± 0.03), and **HCpBA** (R = 0.39 ± 0.04). Scale bar represents 40 μm.

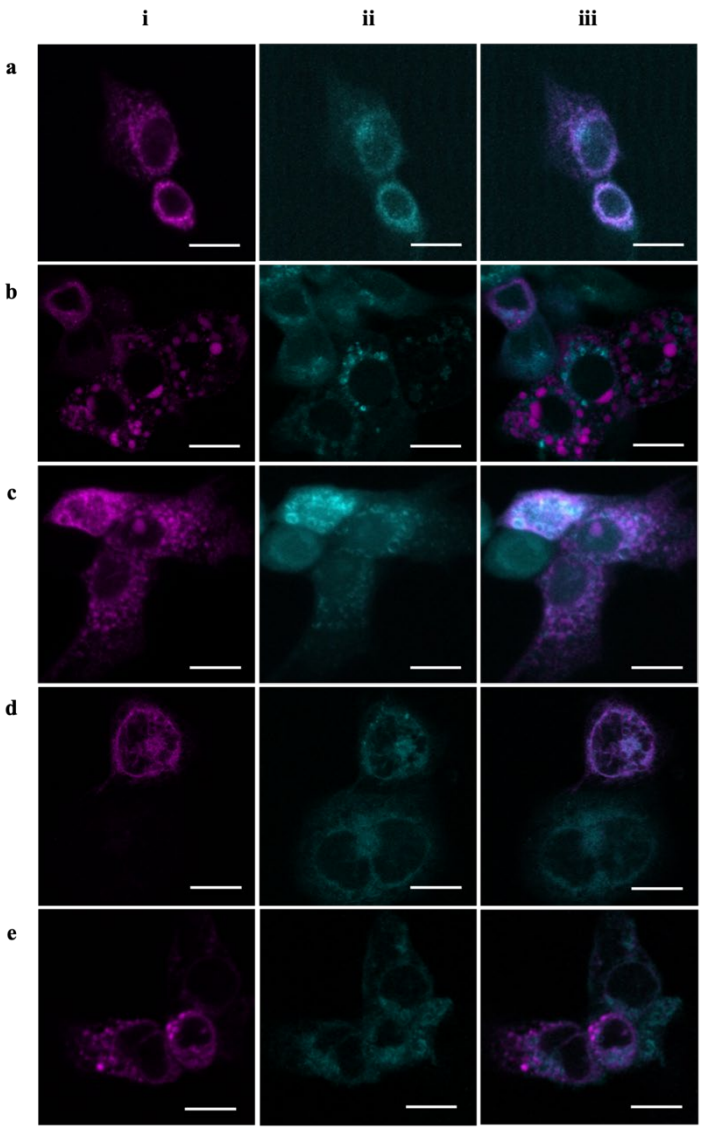

Figure S5: Confocal microscopy images of DLD-1 cells transfected with mCherry-ER and treated with (a) **HCoBA** (10 μM, 20 min), (b) **HCmBA** (10 μM, 20 min), (c) **HCpBA** (10 μM, 20 min), (d) **HCCb** (10 μM, 20 min), or (e) **ICCb** (10 μM, 20 min), showing (i) mCherry-ER fluorescence (λ_{ex} = 561 nm, λ_{em} = 581 - 653 nm), (ii) coumarin fluorescence (λ_{ex} = 458 nm, λ_{em} = 468 - 568 nm), and (iii) overlay of (i) and (ii). Pearson's correlation coefficients observed: **HCoBA** (R = 0.67 ± 0.03), **HCmBA** (0.60 ± 0.07), **HCpBA** (R = 0.73 ± 0.07), **HCCb** (R = 0.47 ± 0.03), and **ICCb** (R = 0.41 ± 0.05). Scale bar represents 20 μm.

Fluorescence Spectroscopy

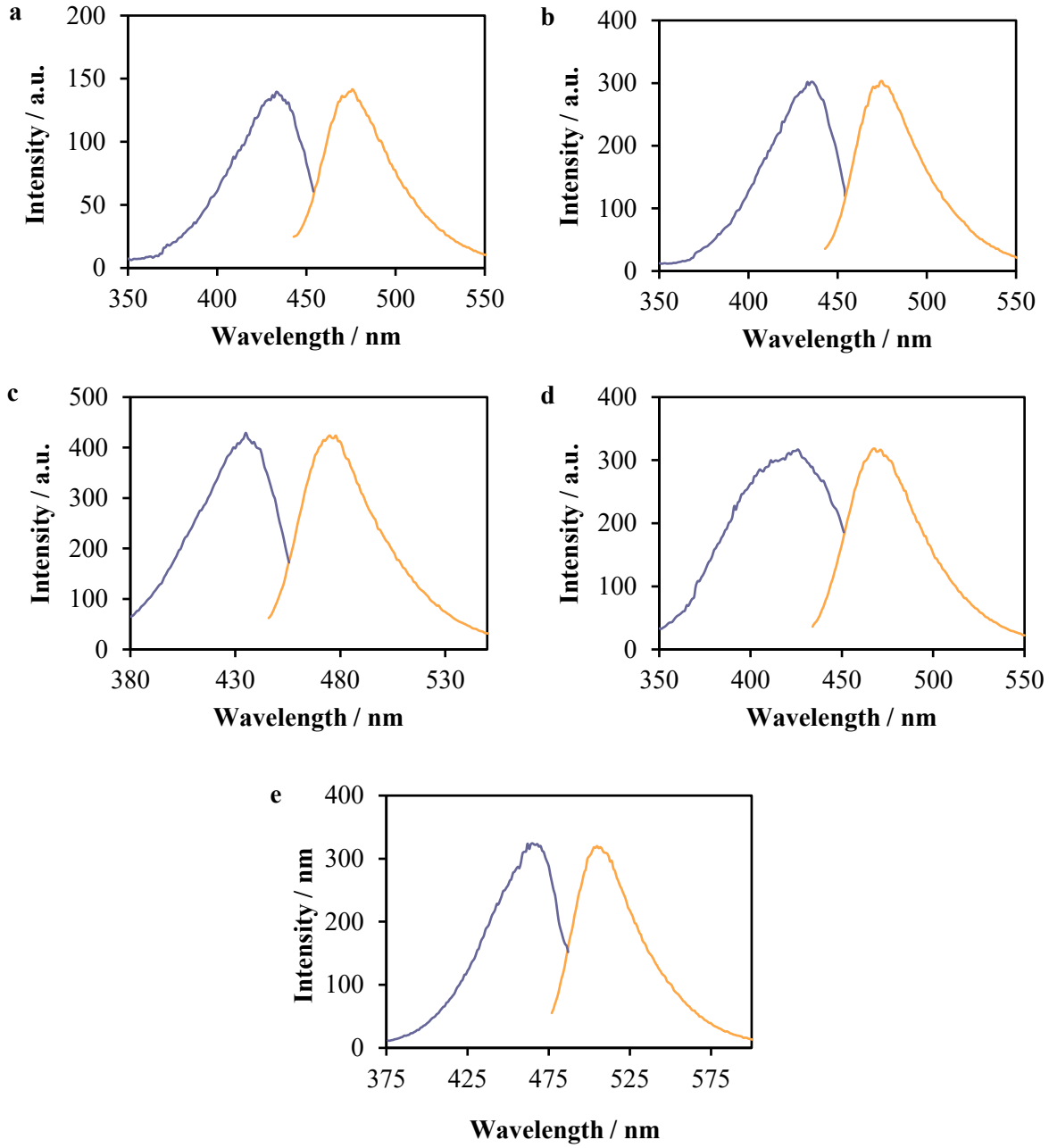

Figure S6: Excitation (blue) and emission (orange) spectra for the boron-based coumarins: (a) **HCoBA** (1 μM, λ_{ex} = 433 nm), (b) **HCmBA** (1 μM, λ_{ex} = 433 nm), (c) **HCpBA** (1 μM, λ_{ex} = 435 nm), (d) **HCCb** (1 μM, λ_{ex} = 425 nm), (e) **ICCb** (1 μM, λ_{ex} = 465 nm).

NMR and MS Spectra of Novel Compounds

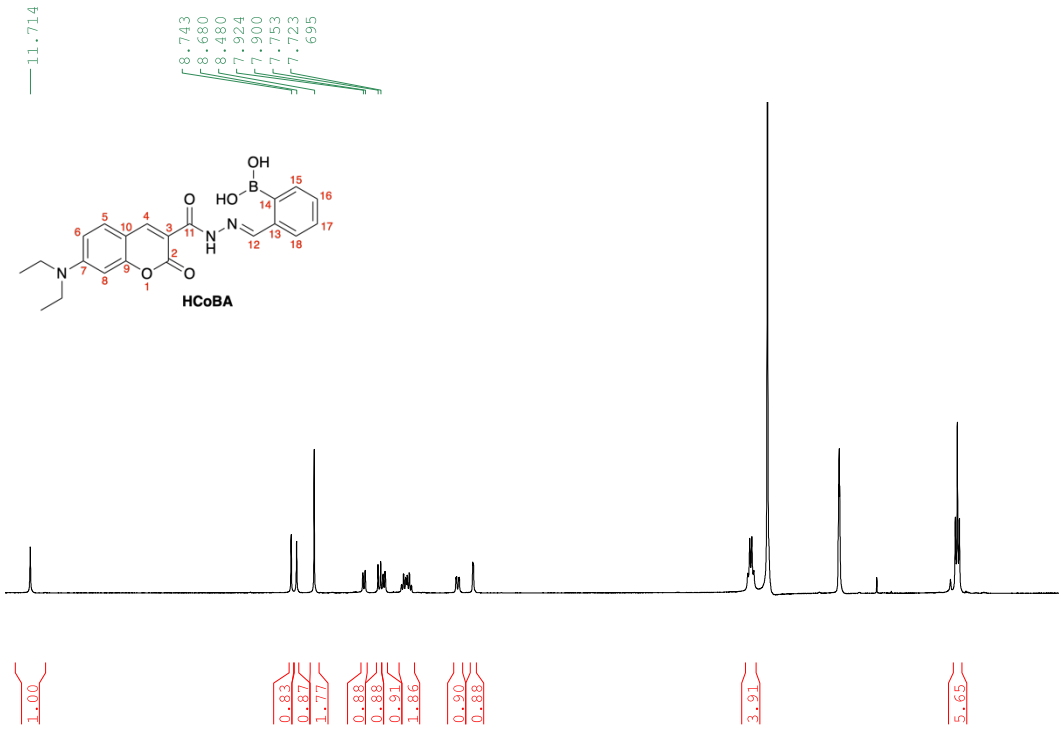

Figure S7.1: ^{1}H NMR spectrum of **HCoBA** in DMSO-d_6.

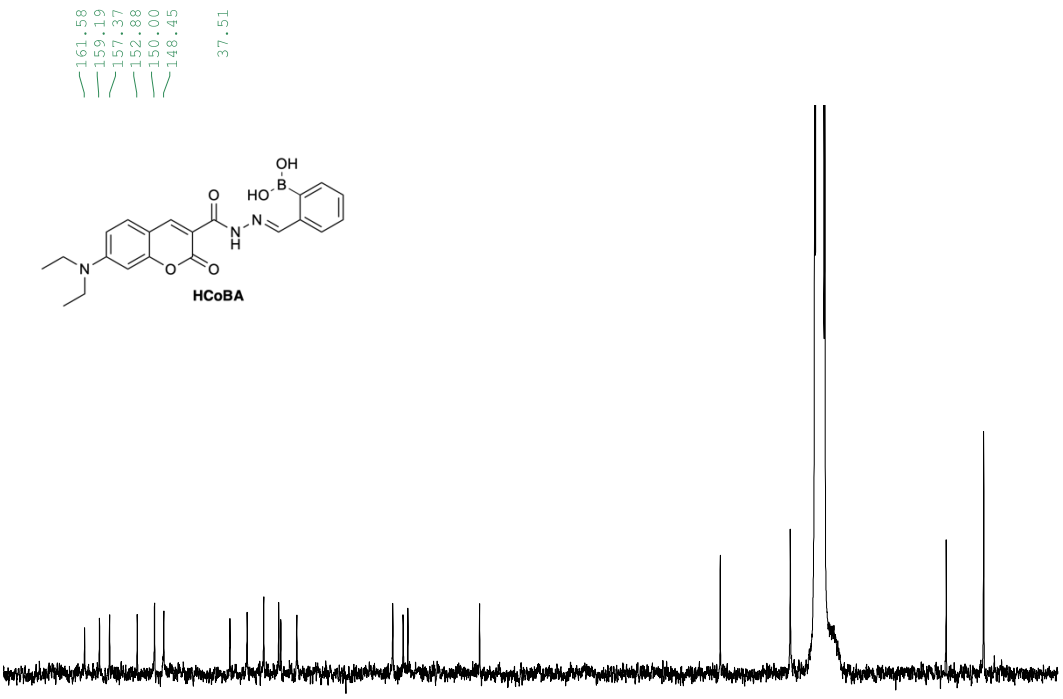

Figure S7.2: ^{13}C NMR spectrum of **HCoBA** in DMSO-d_6.

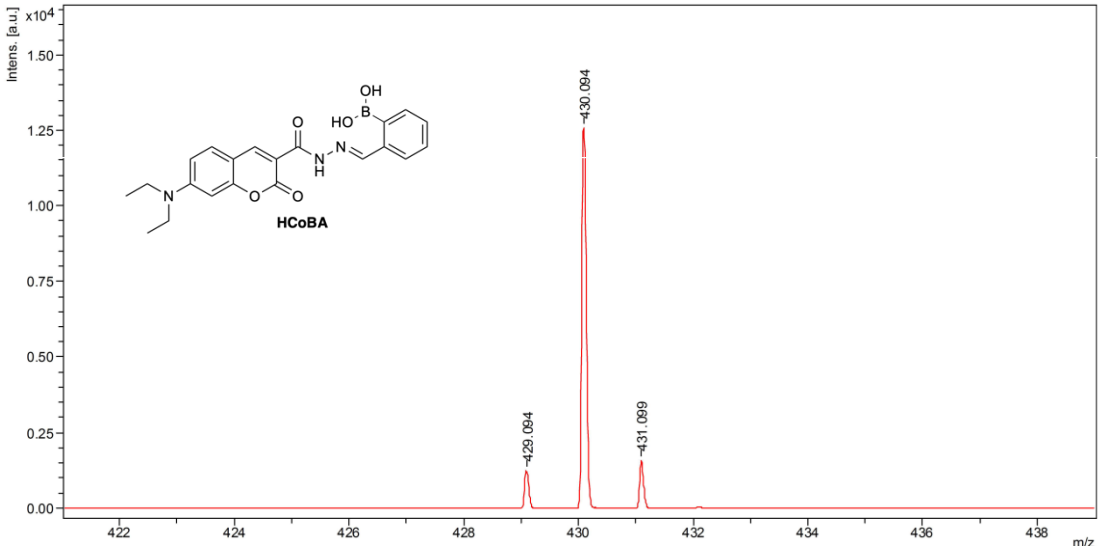

Figure S7.3: MALDI-TOF mass spectrum of **HCoBA**.

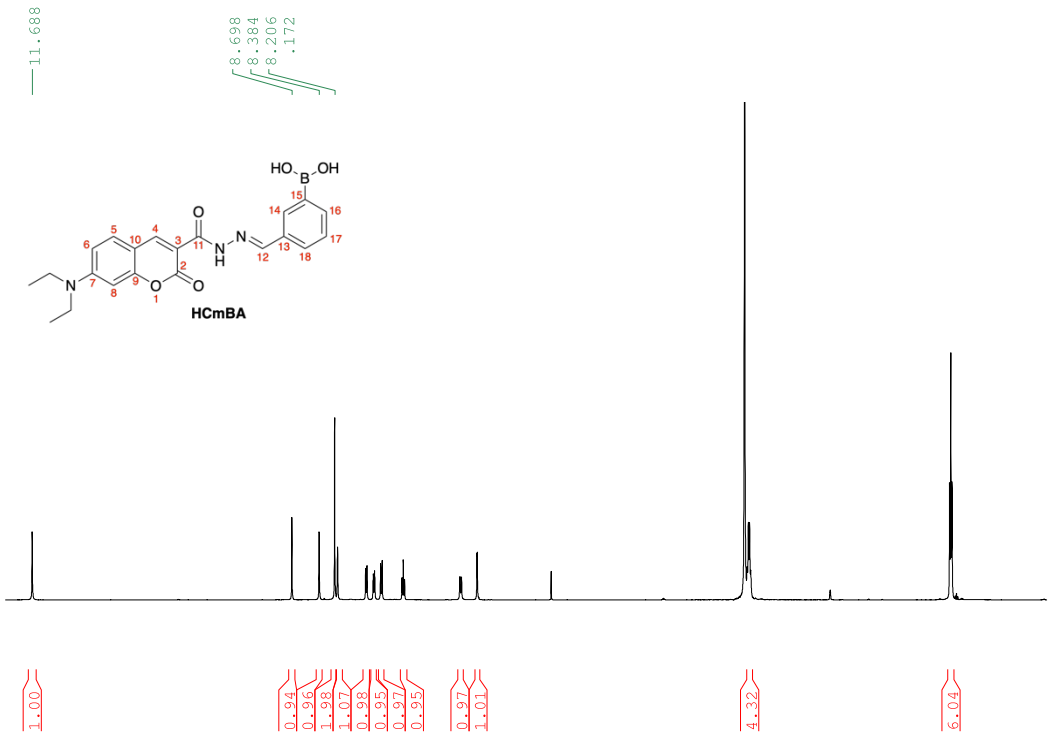

Figure S8.1: ^{1}H NMR spectrum of **HCmBA** in DMSO-d_6.

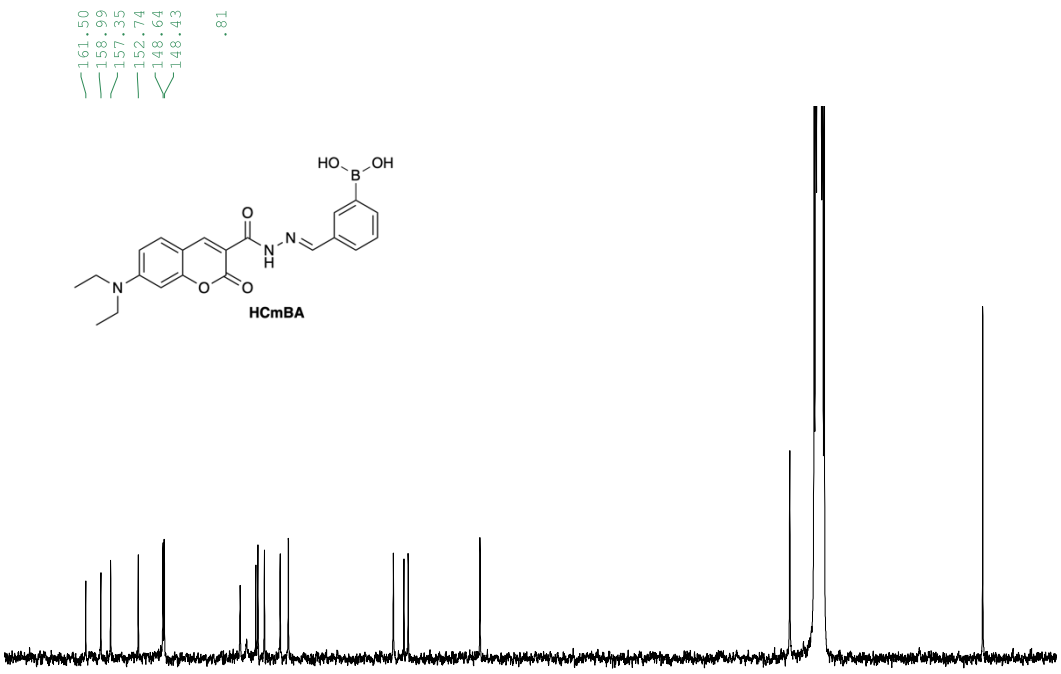

Figure S8.2: ^{13}C NMR spectrum of **HCmBA** in DMSO-d_6.

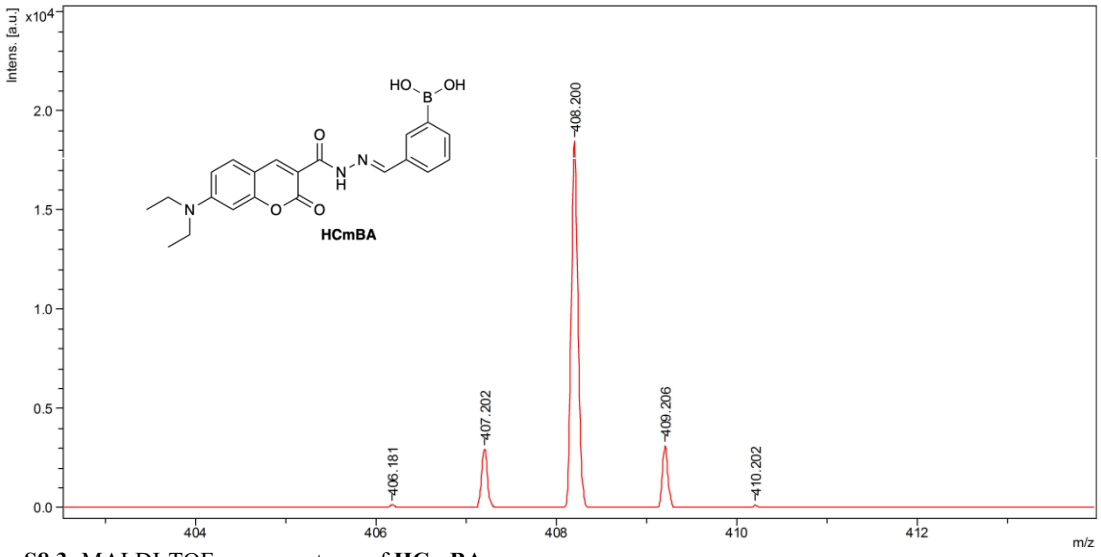

Figure S8.3: MALDI-TOF mass spectrum of **HCmBA**.

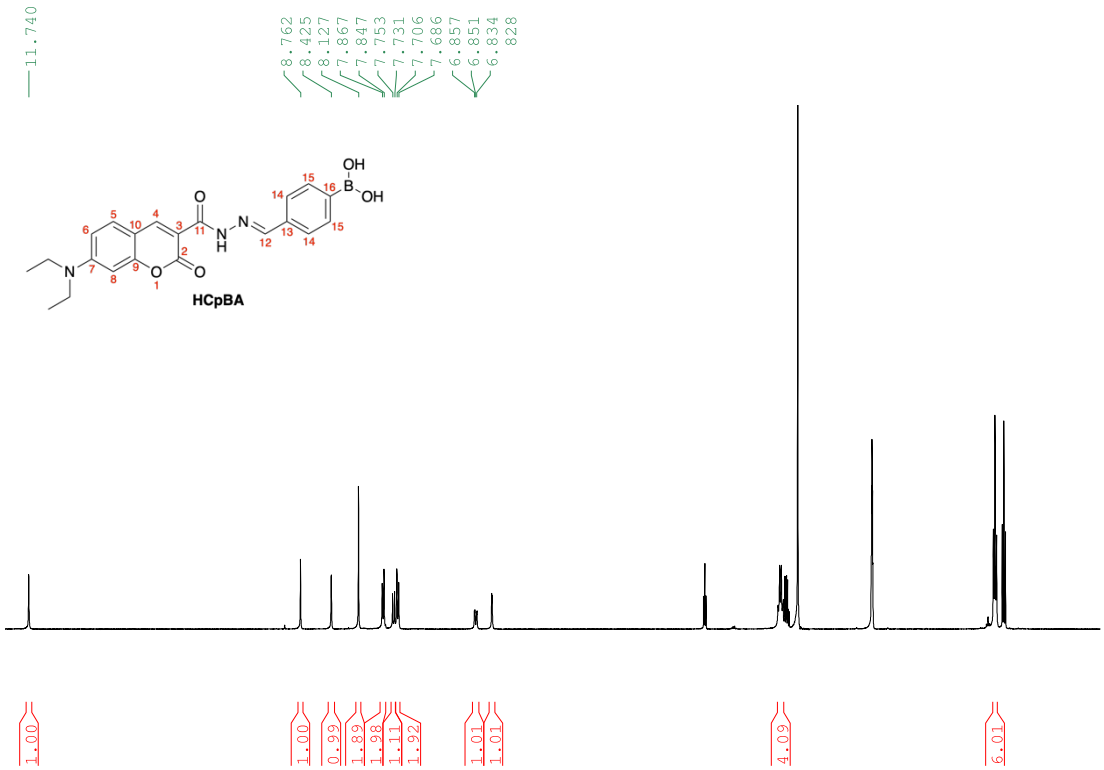

Figure S9.1: ^{1}H NMR spectrum of **HCpBA** in DMSO-d_6.

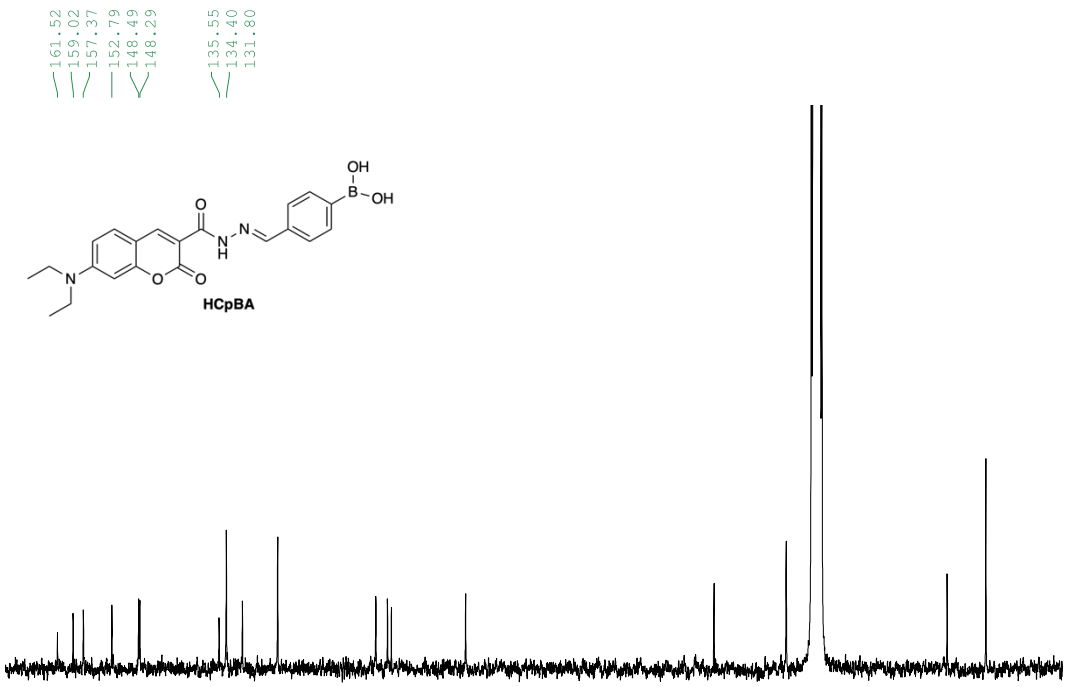

Figure S9.2: ^{13}C NMR spectrum of **HCpBA** in DMSO-d_6.

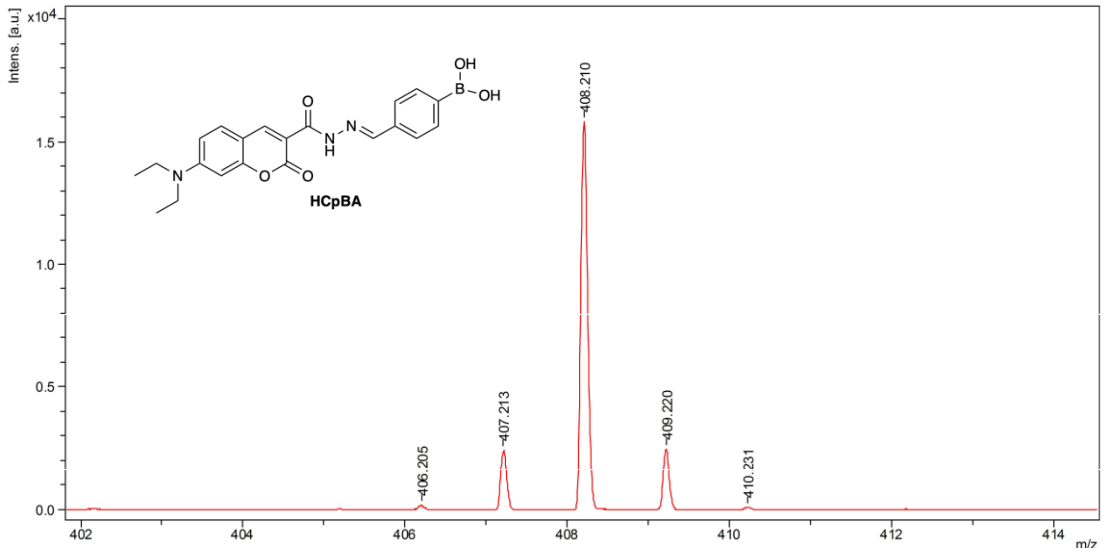

Figure S9.3: MALDI-TOF mass spectrum of **HCpBA**.

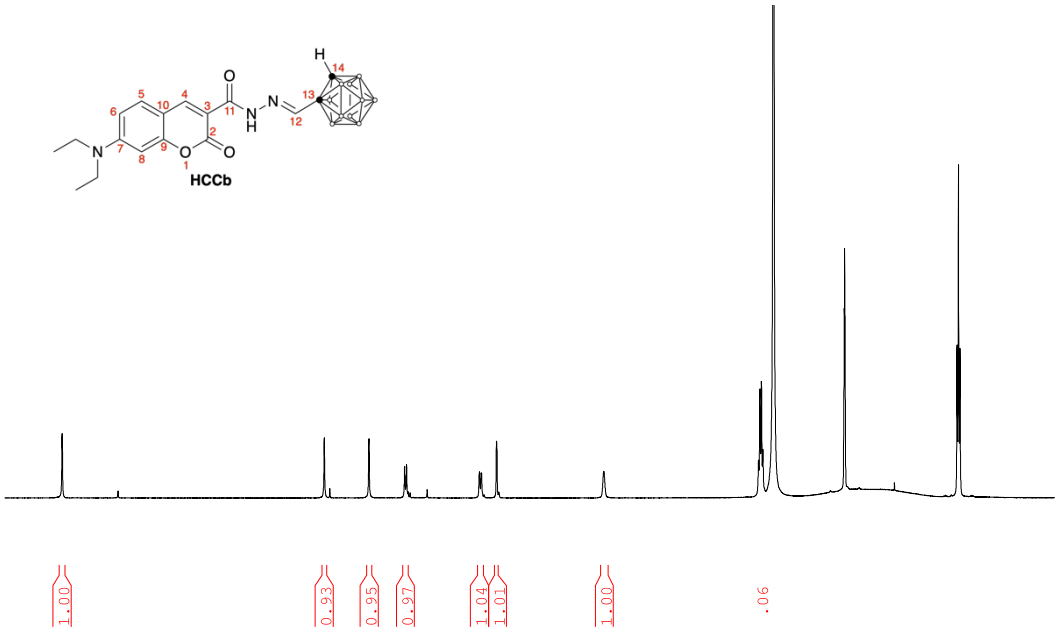

Figure S10.1: ^{1}H NMR spectrum of **HCCb** in DMSO-d_6

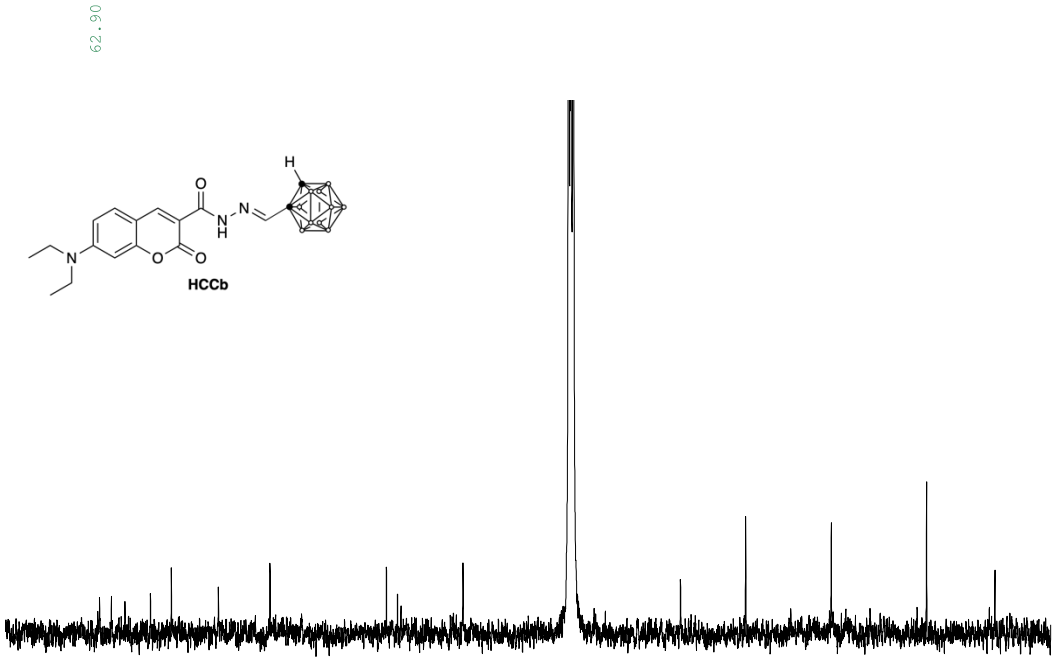

Figure S10.2: ^{13}C NMR spectrum of **HCCb** in $CDCl_3$.

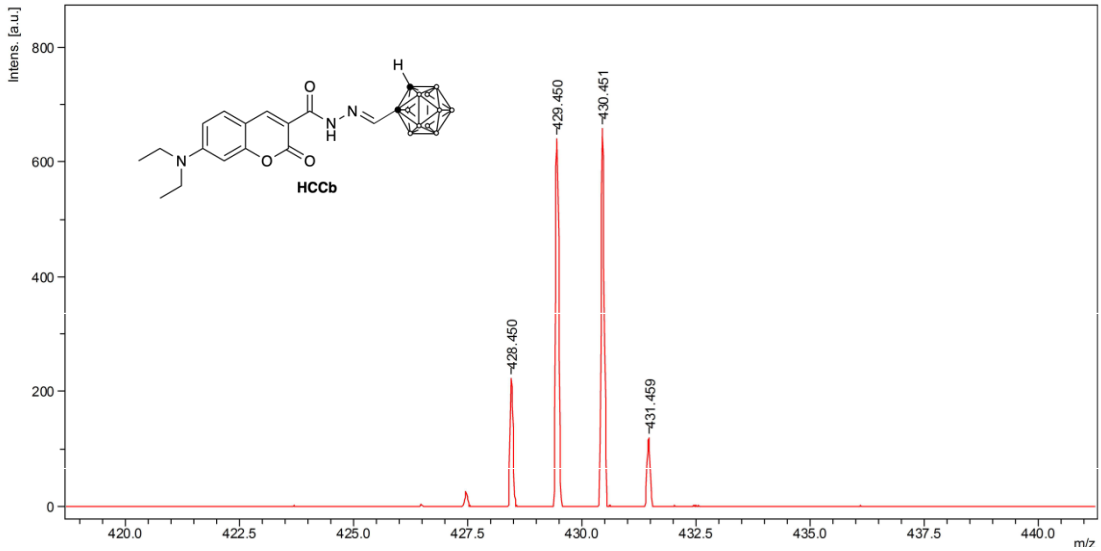

Figure S10.3: MALDI-TOF mass spectrum of **HCCb**.

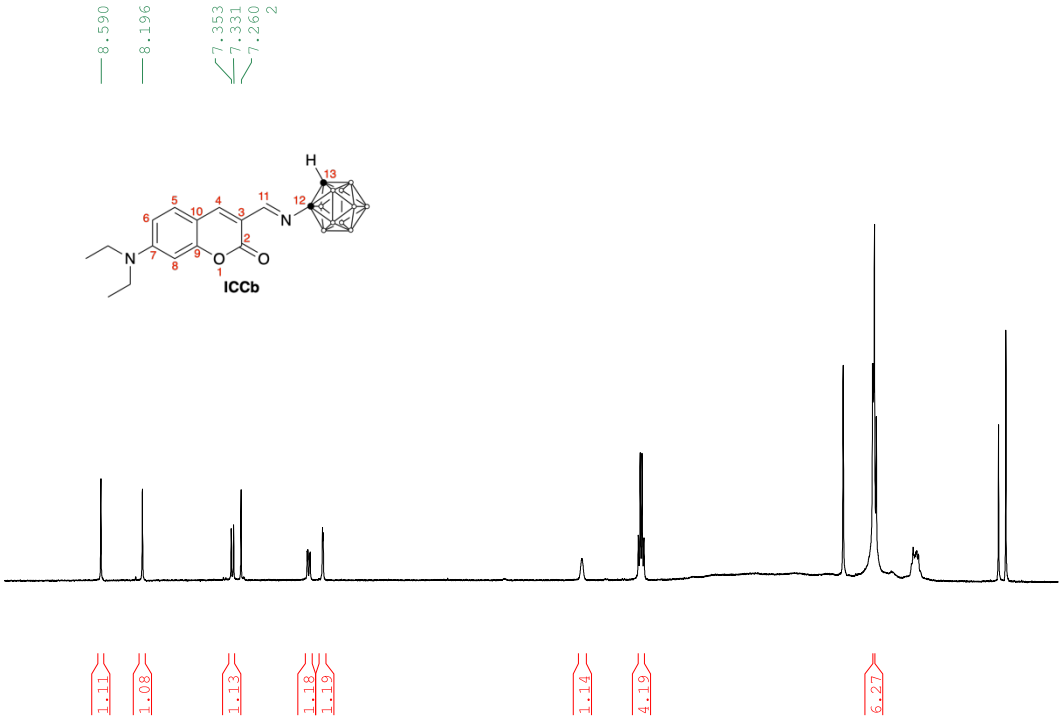

Figure S11.1: ^{1}H NMR spectrum of **ICCb** in $CDCl_3$.

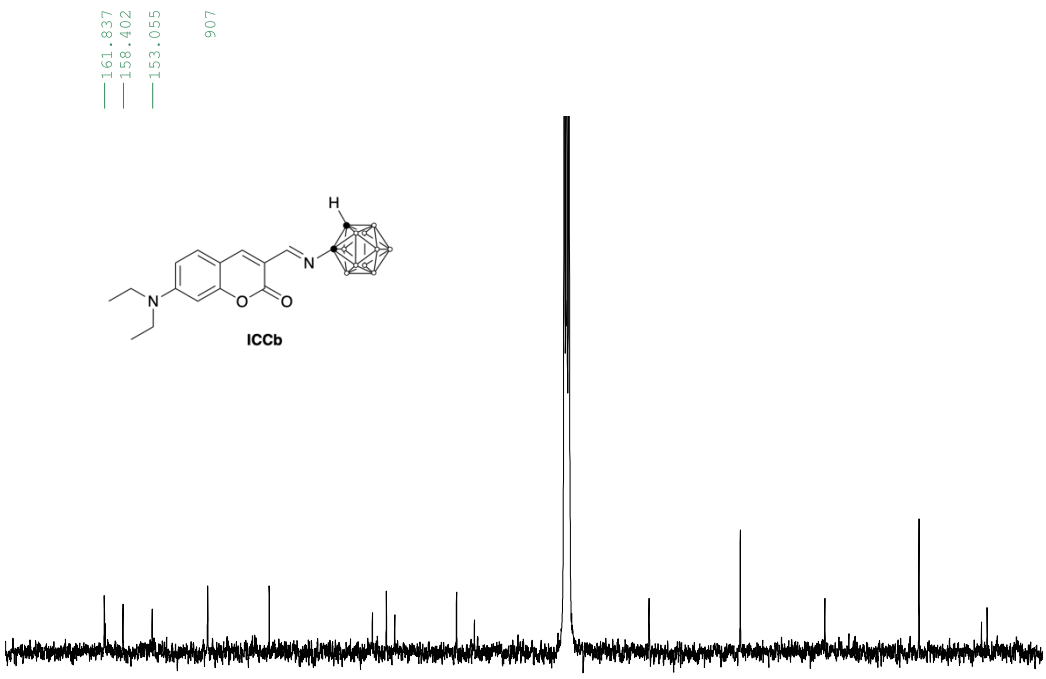

Figure S11.2: ^{13}C NMR spectrum of **ICCb** in $CDCl_3$.

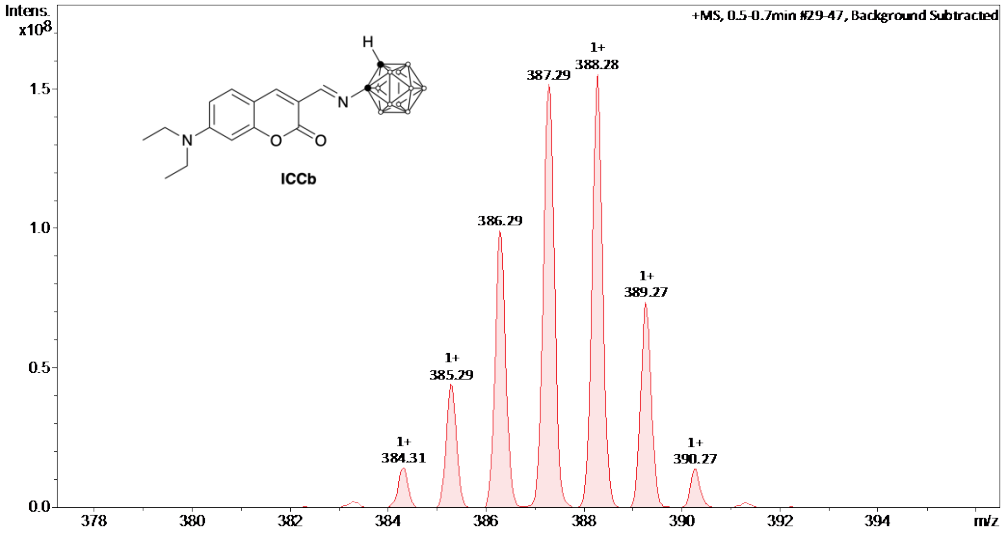

Figure S11.3: APCI mass spectrum of **ICCb**.