Supporting Information

Halogen Bonding BODIPY-Appended Pillar[5]arene for the Optical Sensing of Dicarboxylates and a Chemical Warfare Agent Simulant

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1. Instrumentation and General Experimental Details

General Information

Solvents and reagents were purchased from commercial suppliers and used as received. Dry solvents were obtained by purging with nitrogen and passing through a MBraun MPSP-800 column. H_2O was de-ionised and micro-filtered using a Milli-Q® Millipore machine.

Experiments were conducted at room temperature unless otherwise stated. Merck silica gel 60 was used for flash column chromatography. TBA salts were stored in vacuum desiccators prior to use. NMR spectra were either recorded on a Bruker Avance III HD Nanobay NMR spectrometer equipped with a 9.4 T magnet or a Bruker NEO 600 with broadband helium

cryoprobe. ^{1}H NMR titrations were recorded on a Bruker Avance III NMR equipped with a 11.75 T magnet.

Chemical shifts are quoted in parts per million relative to the residual solvent peak.

UV-vis and fluorescence measurements were carried out on a Duetta (Horiba) using quartz cuvettes with a path length of 10 mm. Unless otherwise noted, all fluorescence spectra were acquired with a wavelength of excitation of 490 nm, 5 nm excitation and emission slits and were recorded in, at least, triplicate repeat measurements to ensure signal stability. Anion titration studies were carried out by titrating a 1 μM solution of the receptor with aliquots of a concentrated solution of TBA-anion in the same receptor solution to ensure a constant receptor concentration.

$(TBA)_2DCB$ salts, [1] were prepared in line with literature procedures. CEES (98.0%) was purchased from Fluorochem and used as supplied.

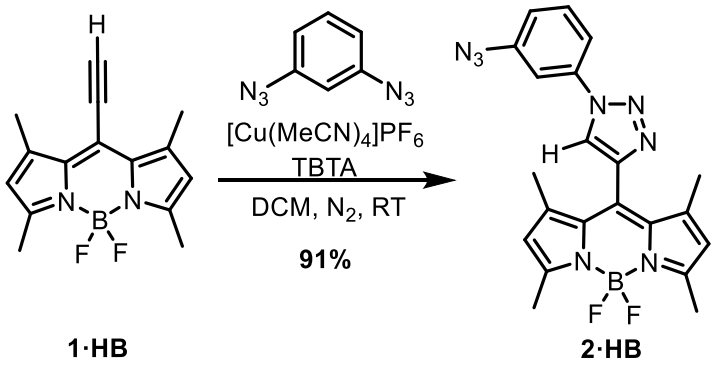
50 mM HEPES buffer was prepared by dissolving HEPES (N-(2-Hydroxyethyl)piperazine-N'-(2-ethanesulfonic acid)) in distilled water and then adjusting the pH to 8.0 by addition of small volumes of a highly concentrated (3M) NaOH solution.

Tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine is abbreviated as TBTA.

All data analysis and fitting were carried out with *OriginPro* 2023.[2]

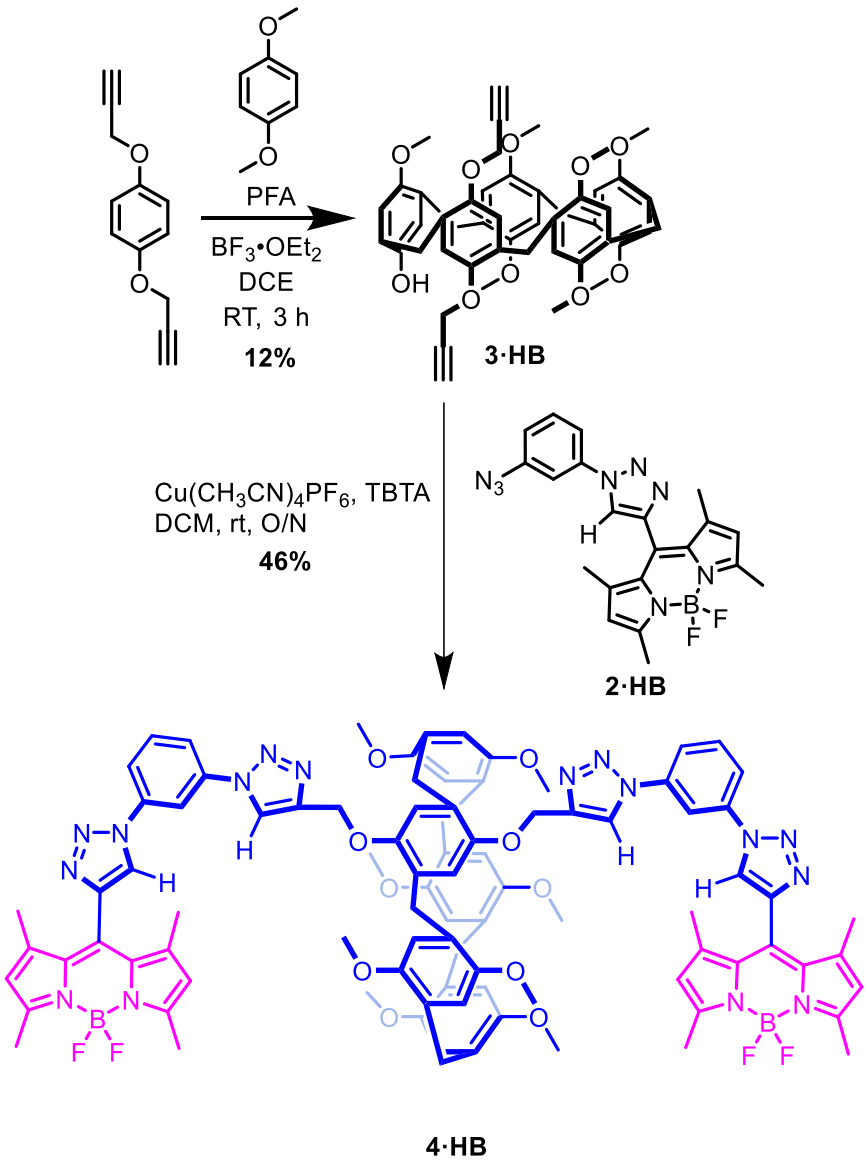
2. Synthesis and Characterisation of Compounds

Synthesis of HB Analogues



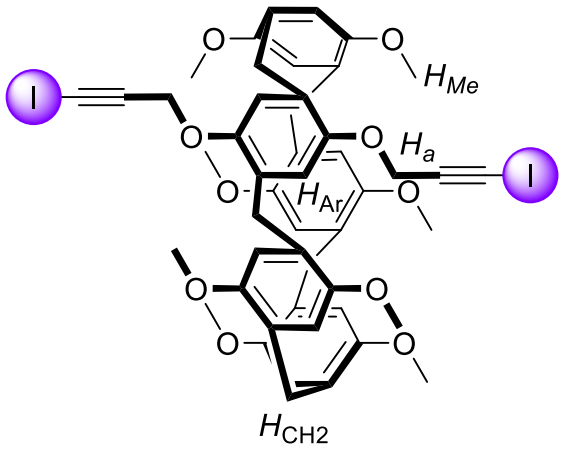
Scheme S1. Synthesis of **2·HB.**

8-Ethynyl-BODIPY[3] was subjected to typical CuAAC ('click') conditions with a large excess 1,3-diazidobenzene,[4-5] producing the mono-click product **2·HB** in 91% yield (Scheme S1). The bis alkyne-functionalised P5A **3·HB** was obtained in moderate yield (12%) by statistical condensation of 1,4-bis(prop-2-yn-1-yloxy)benzene and 1,4-dimethoxybenzene with paraformaldehyde under conventional P5A synthesis conditions.[6] **2·HB** was then re-subjected to CuAAC conditions with **3·HB** to afford title compound **4·HB** in 46% yield (Scheme S2).



Scheme S2. Synthesis of **3·HB** and **4·HB.**

3·XB



3·XB

3·HB (170.0 mg, 0.313 mmol) and catalytic CuI (5.9 mg, 0.031 mmol) were dissolved in dry THF (2 mL). *N*-iodomorpholine hydriodide (420 mg, 1.235 mmol) was added, and the mixture stirred at room temperature, under exclusion of light, for 3 h, after which only one spot was visible by TLC (10% EtOAc *v/v* in hexanes). The reaction mixture was diluted with DCM (50 mL), and poured onto a saturated DCM pad of neutral alumina. The pad was eluted with DCM until the solution ran clear and the organic layer decolourised with saturated $Na_2S_2O_3$ (aq.) (25 mL), and washed with H_2O (3 x 100 mL). The organic layer was dried over $MgSO_4$ and the volatiles removed *in vacuo*. The crude product was dry-loaded onto SiO_2 and eluted with 10% EtOAc *v/v* in hexanes to afford **3·XB** as an off-white solid. The product was estimated by ^{1}H NMR to be 66% pure, with the contaminant identified as non-functionalised *per*-methoxypillar[5]arene, residual from the synthesis of **3·HB**. As the side product would not react in the subsequent step, **3·XB** was used without further purification. Yield: 329.1 mg (66%).

^{1}H NMR (400 MHz, $CDCl_3$) δ: 6.89 6.88 6.85 6.80 6.76 (5 s, 2H each, H_{Ar}), 4.79 (s, 4 H, H_a), 3.72 (m, 10H, H_{CH2}), 3.69-3.61 (m, 24H, H_{Me}) ppm.

^{13}C{^{1}H} NMR (151 MHz, $CDCl_3$) δ: 150.6, 150.4, 150.3, 148.7, 129.4, 128.7, 128.0, 127.7, 127.6, 116.2, 114.3, 113.8, 113.7, 113.4, 89.5, 57.7, 55.8, 55.7, 55.6, 55.5, 29.8, 29.7, 29.6, 29.0 ppm. Iodoalkyne environment is not observed, and is likely coincident with the $CDCl_3$ solvent resonance.

HR-ESI-MS: m/z calculated for $[C_{49}H_{48}I_2O_{10}Na]^+$, $[M+Na]^+$: 1073.1229, found: 1073.1272.

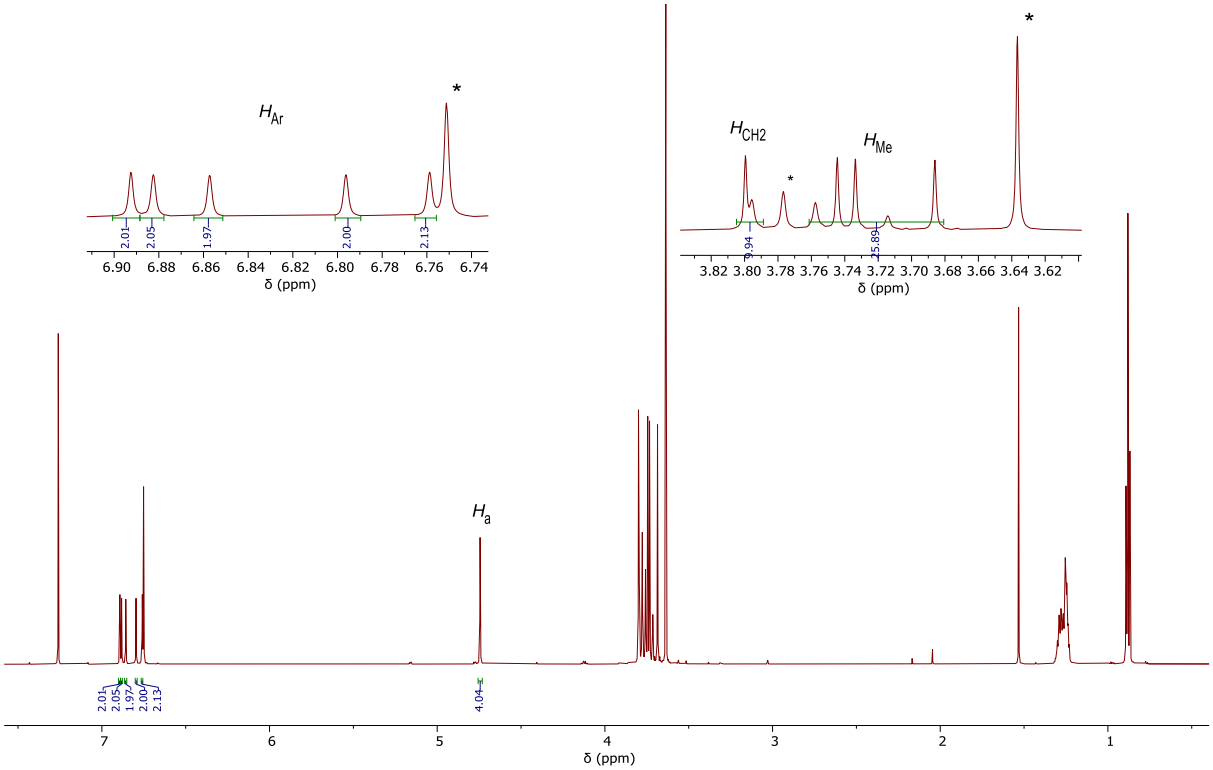


Figure S1. ^{1}H NMR spectrum of **3·XB.** Asterisks indicate signals arising from permethylated P5A impurity.

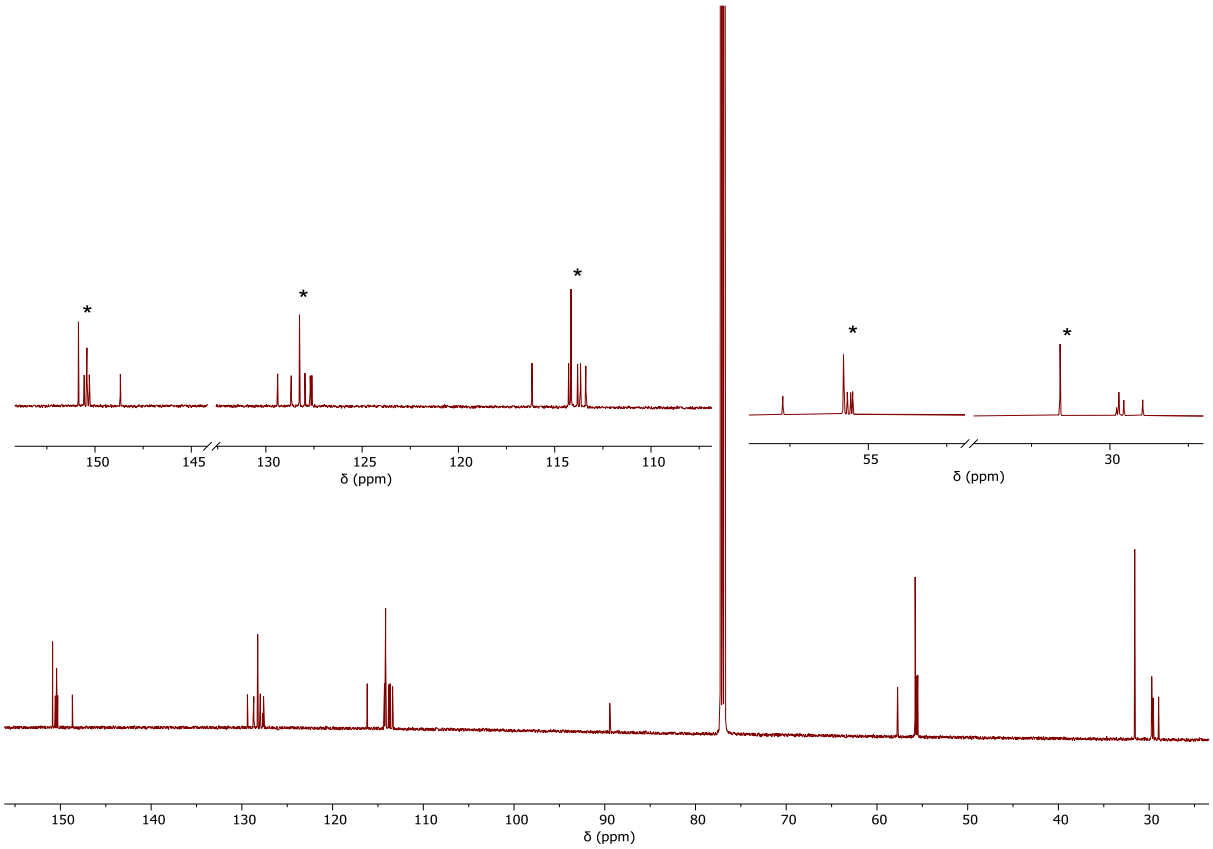
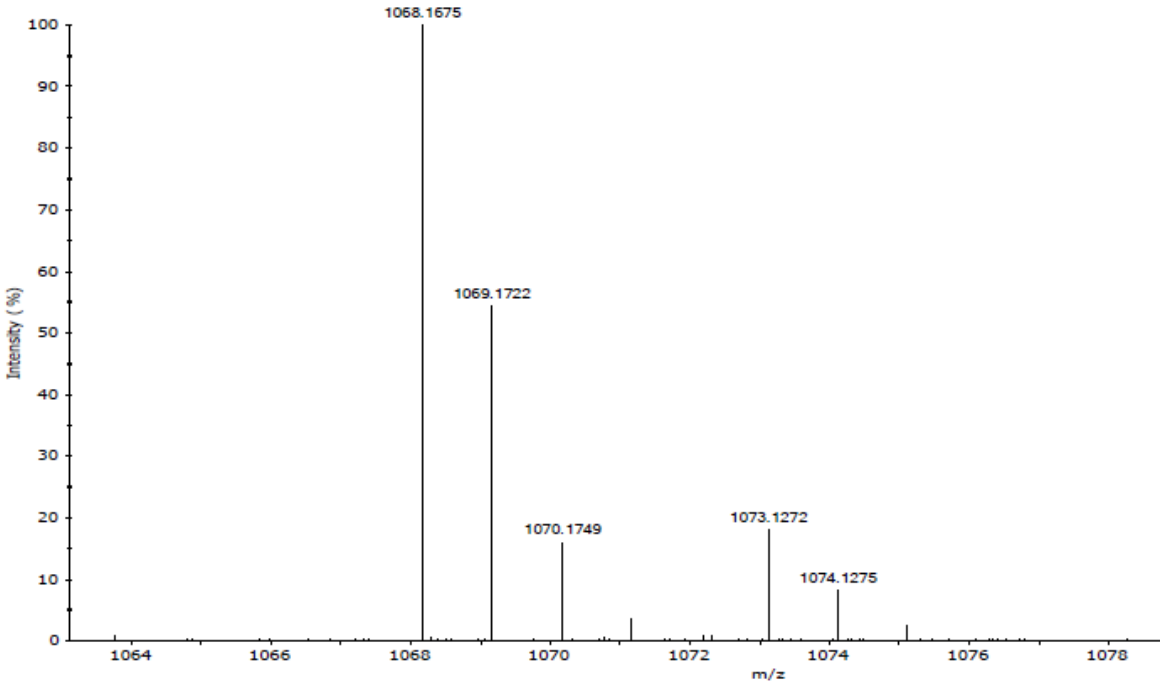


Figure S2. ^{13}C NMR spectrum of **3·XB.** Asterisks indicate signals arising from permethylated P5A impurity.



Theoretical Spectrum for C49H48I2O10Na, Minimum Abundance 0.01%

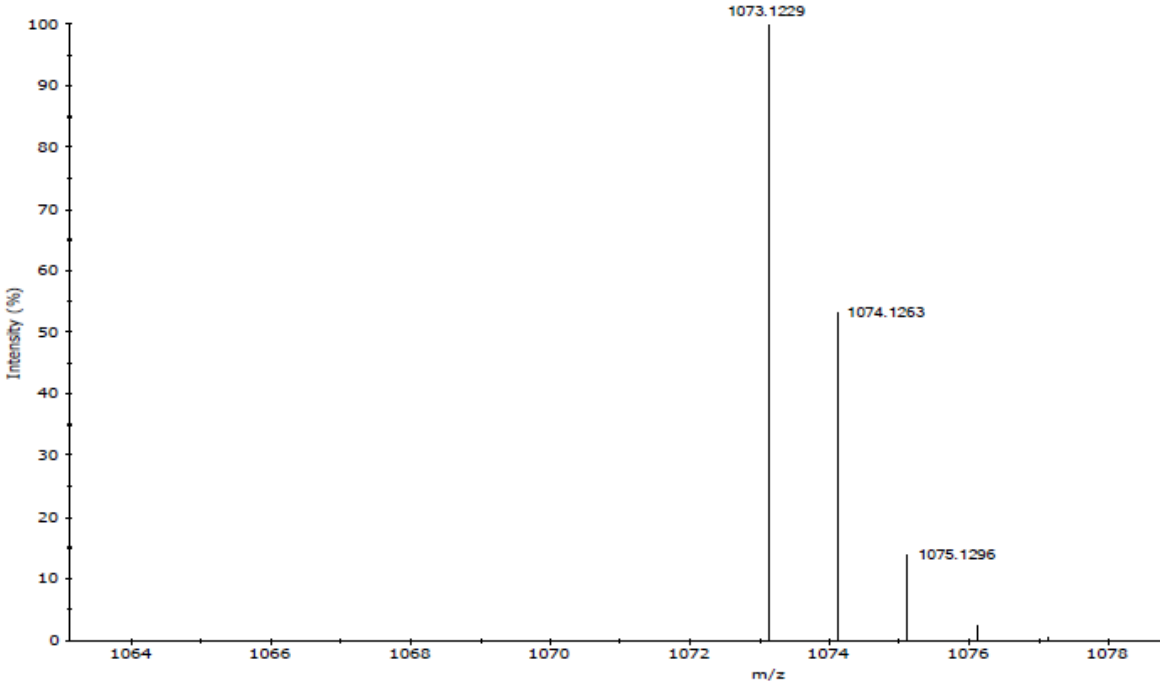
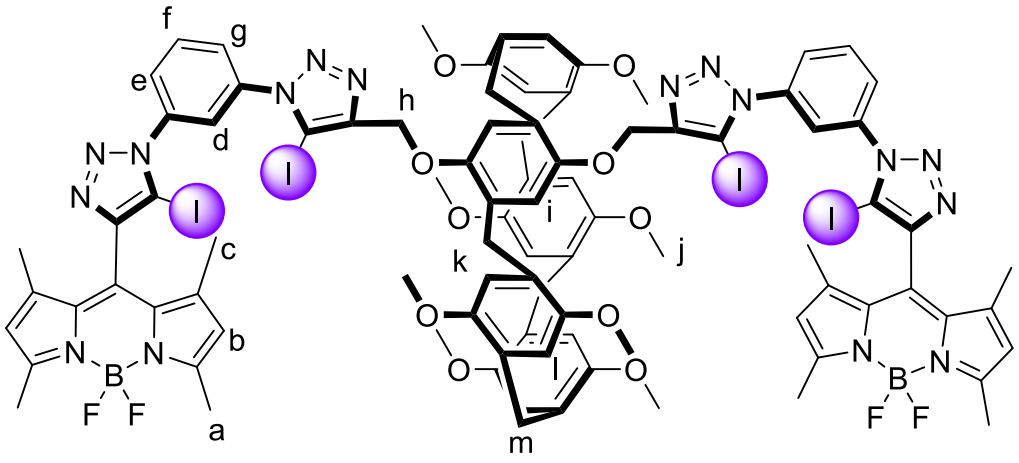


Figure S3. Experimental and theoretical mass spectra of **3·XB.**

4·XB



4·XB

$[Cu(MeCN)_4]PF_6$ (5.6 mg, 0.015 mmol) and TBTA (8.0 mg, 0.015 mmol) were placed in a 5 mL microwave vial and degassed for 10 minutes. The solids were dissolved in the minimum amount of degassed dry DCM (3 mL) and stirred at room temperature for 15 min. XB BODIPY precursor **2·XB** (40 mg, 0.072 mmol) and P5A bis-alkyne **3·XB** (18.9 mg, 0.018 mmol) were dissolved in the minimum amount of degassed dry DCM (2 mL) and the resulting solution added to the pre-complexed Cu^{I}/TBTA solution. The resulting solution was stirred at room temperature, under exclusion of light, for 12 h, after which the reaction mixture was diluted with DCM (15 mL) and washed with NH_4OH/EDTA (aq.) (2 x 20 mL) and H_2O (20 mL). All aqueous layers were back-extracted with DCM (10 mL) and the combined organic layers were dried over $MgSO_4$ and the volatiles removed *in vacuo*. **4·XB** was obtained by column chromatography of the resulting crude solid in acetone:DCM mixtures graded from *v/v* 0:100 to 3:97, as a purple solid. Yield 28.0 mg (72%).

^{1}H NMR (400 MHz, Acetone-d_6) δ: 8.17 (broad s, 2H, H_f), 8.08 (app. s, 6H, $H_{d,e,g}$), 7.23 (s, 2H, H_i), 6.91-6.86 (m, 8H, H_l), 6.23 (s, 4H, H_b), 5.16 (app. t, 4H, H_h), 3.81-3.64 (m, 34H, $H_{j,k,m}$), 2.55 (s, 12H, H_c), 1.64 (s, 12H, H_a) ppm.

^{13}C{^{1}H} NMR (151 MHz, Acetone-d_6) δ: 157.2, 150.5, 150.5, 150.4, 150.4, 150.0, 148.8, 146.4, 142.7, 138.1, 137.5, 132.1, 131.1, 129.4, 128.4, 128.3, 128.2, 128.2, 128.1, 128.0,

127.8, 124.3, 121.8, 121.8, 115.9, 113.7, 113.5, 113.4, 85.2, 83.5, 62.7, 55.3, 55.1, 55.1, 55.0, 13.9, 13.2 ppm.

HR-ESI-MS: m/z calculated for $[C_{91}H_{85}B_2F_4I_4N_{16}O_{10}]^+$, $[M+H]^+$: 2167.2930, found: 2167.2886.

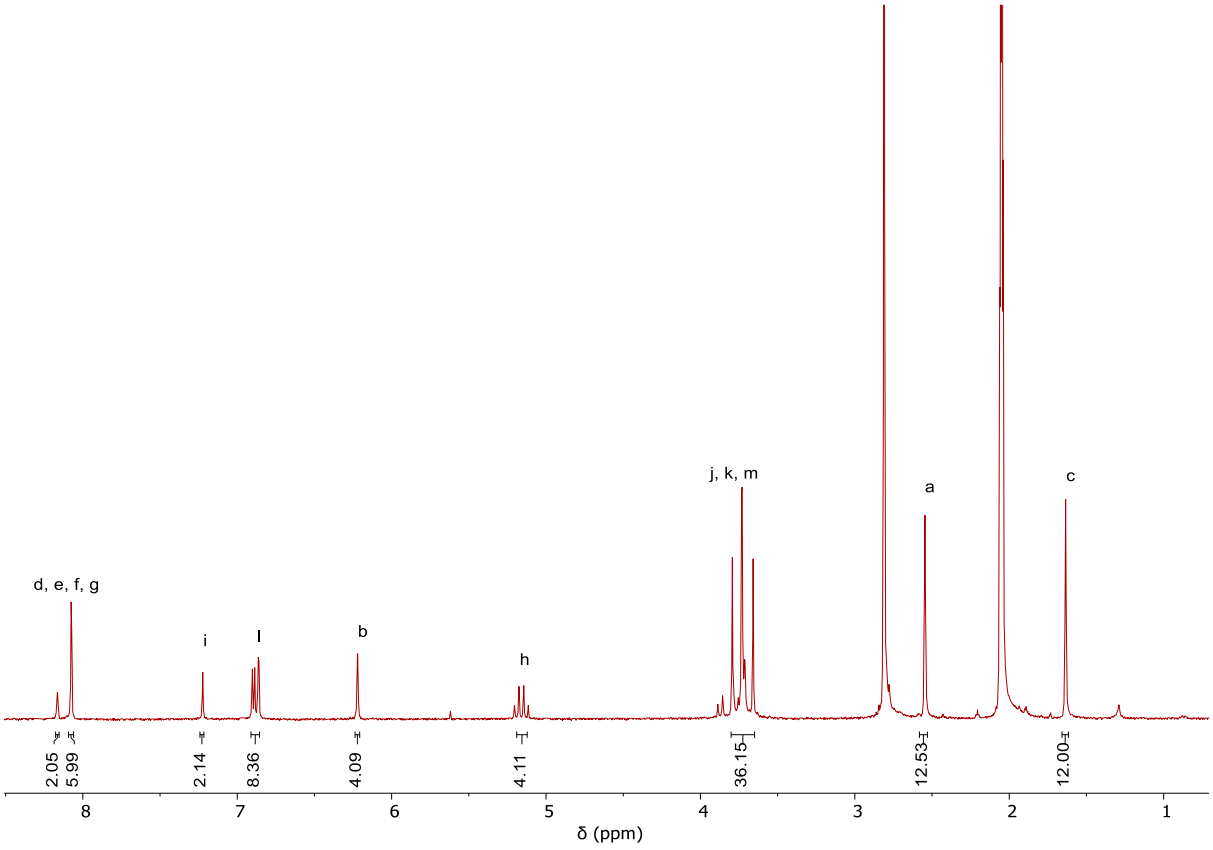


Figure S4. ^{1}H NMR spectrum of **4·XB.**

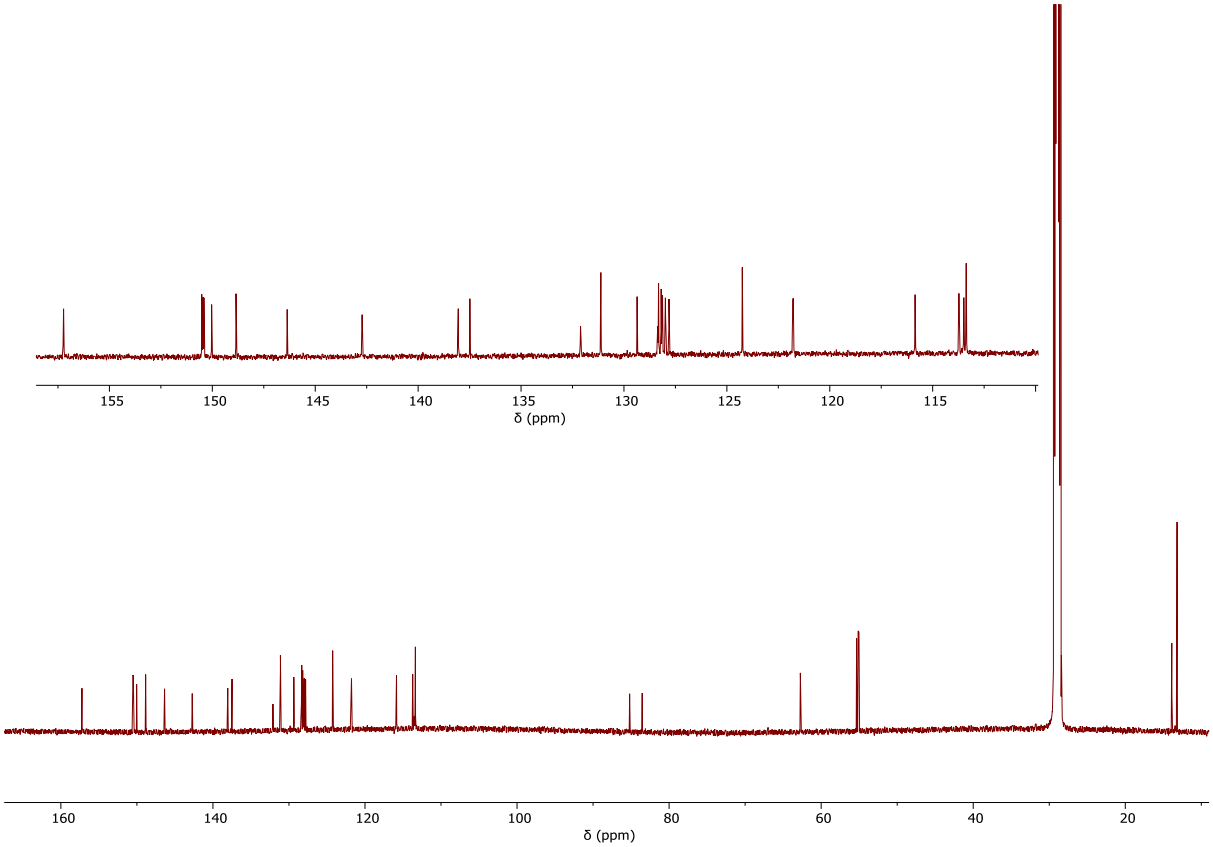


Figure S5. ^{13}C NMR spectrum of **4·XB.**

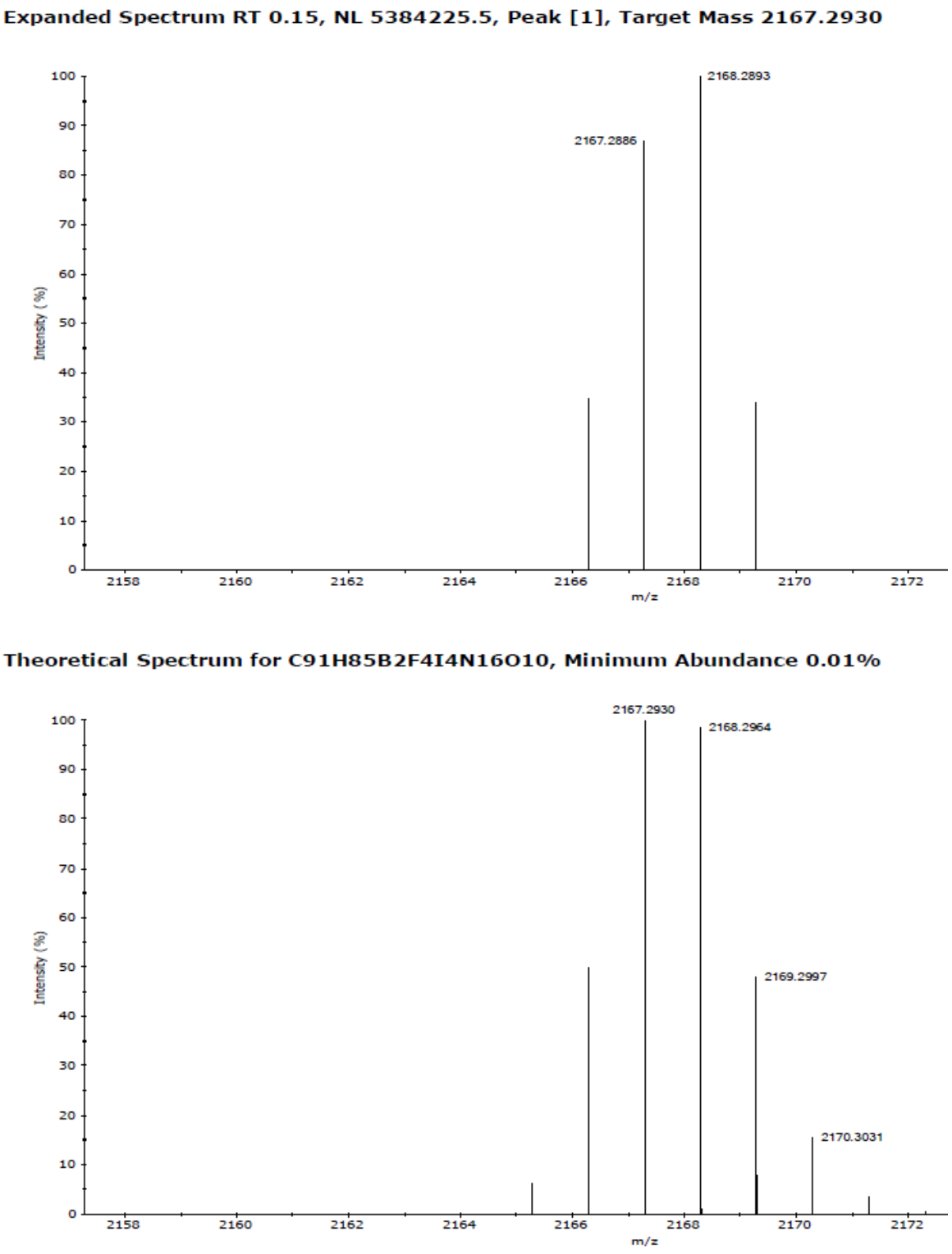
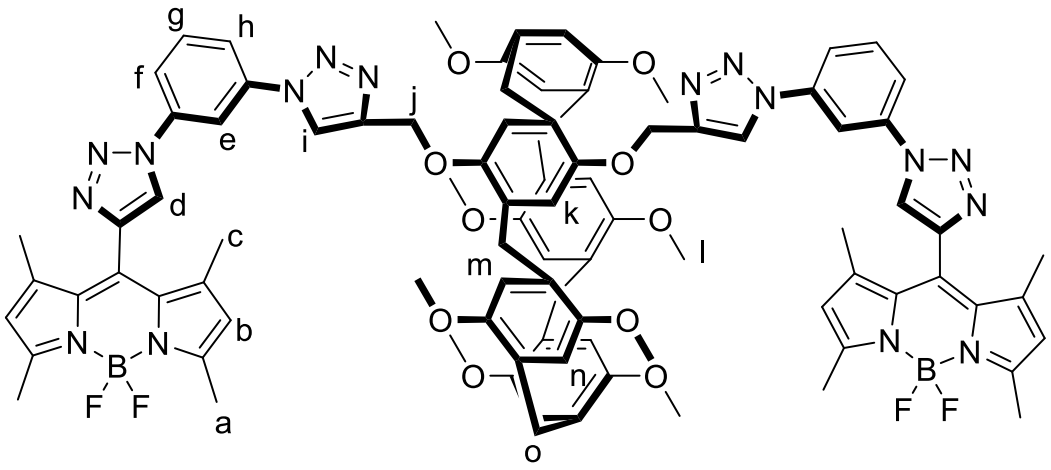


Figure S6. Experimental and theoretical mass spectra of **4·XB.**

4·HB



4·HB

$[Cu(MeCN)_4]PF_6$ (5.0 mg, 0.013 mmol) and TBTA (7.1 mg, 0.013 mmol) were placed in a 5 mL microwave vial and degassed for 10 minutes. The solids were dissolved in the minimum amount of degassed dry DCM (3 mL) and stirred at room temperature for 15 min. HB BODIPY precursor **2·HB** (25.0 mg, 0.058 mmol) and P5A bis-alkyne **3·HB** (12.0 mg, 0.015 mmol) were dissolved in the minimum amount of degassed dry DCM (2 mL) and the resulting solution added to the pre-complexed Cu^{I}/TBTA solution. The resulting solution was stirred at room temperature for 12 h, after which the reaction mixture was diluted with DCM (15 mL) and washed with NH_4OH/EDTA (aq.) (2 x 20 mL) and H_2O (20 mL). All aqueous layers were back-extracted with DCM (10 mL) and the combined organic layers were dried over $MgSO_4$ and the volatiles removed *in vacuo*. **4·HB** was obtained by column chromatography of the resulting crude solid in acetone:DCM mixtures graded from *v/v* 0:100 to 3:97, as a purple solid. Yield 24.2 mg (46%).

^{1}H NMR (400 MHz, Acetone-d_6) δ: 9.08 8.96 (2s, 2H each, $H_{d,i}$), 8.64 (t, J = 2.1 Hz, 2H, H_e), 8.17 (m, 4H, $H_{f,h}$), 7.94 (t, J = 8.2 Hz, H_g), 7.13 (s, 2H, H_k), 6.87-6.84 (m, 8H, H_n), 6.18 (broad s, 4H, H_b), 5.20 (m, 4H, H_j), 3.76-3.59 (m, 34H, $H_{l,o}$), 2.53 (s, 12H, H_c),1.62 (s, 12H, H_a) ppm.

^{13}C{^{1}H} NMR (151 MHz, Acetone-d_6) δ: 156.6, 150.5, 150.5, 150.5,149.8,145.7, 143.1, 140.6, 138.4, 138.1, 132.2, 131.7, 129.2, 128.9, 128.8, 128.2, 128.2, 128.1, 128.1, 128.1, 128.0,

127.9, 127.7, 123.4, 122.0, 120.1, 115.1, 113.7, 113.5, 113.4, 112.2, 75.4, 62.2, 55.1, 55.1, 55.1, 55.0, 13.6, 12.9 ppm.

HR-ESI-MS: m/z calculated for $[C_{91}H_{89}B_2F_4N_{16}O_{10}]^+$, $[M+H]^+$: 1663.7064, found: 1663.7053.

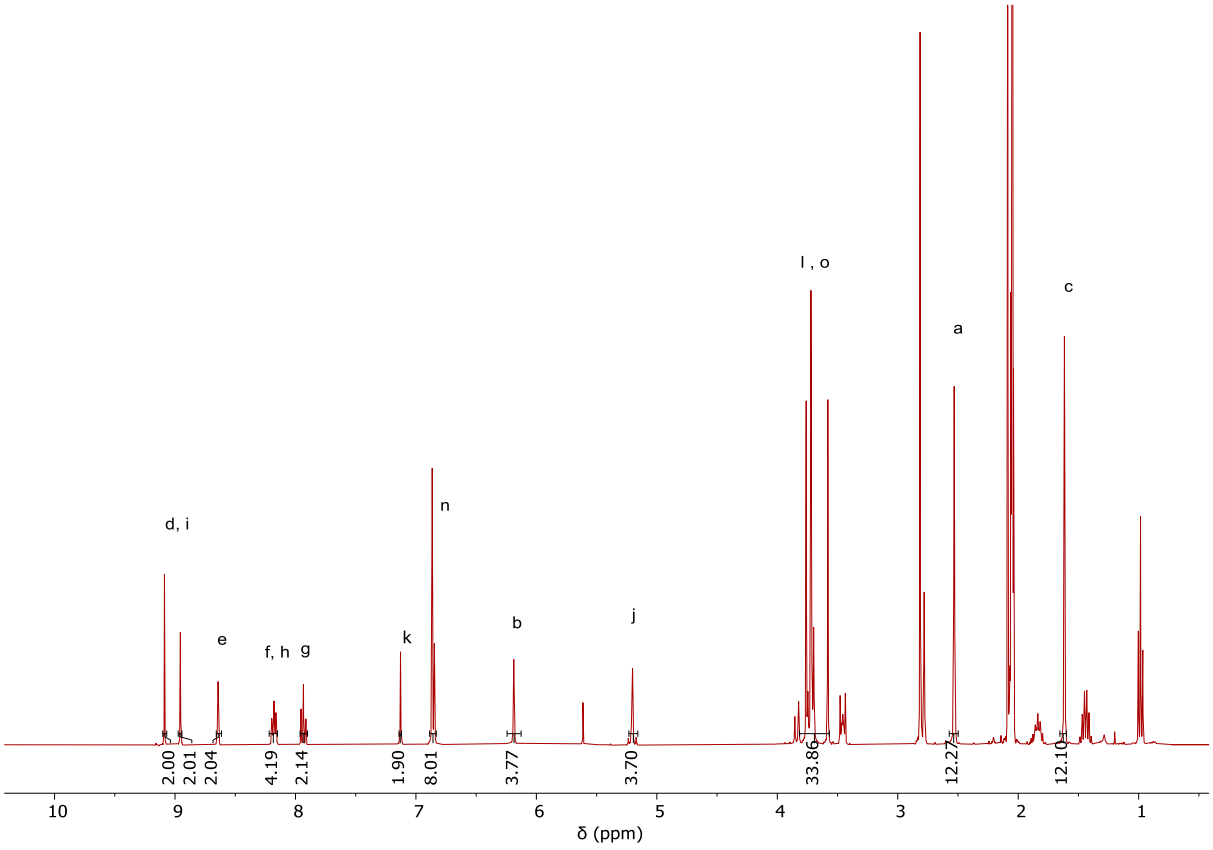


Figure S7. ^{1}H NMR spectrum of **4·HB.**

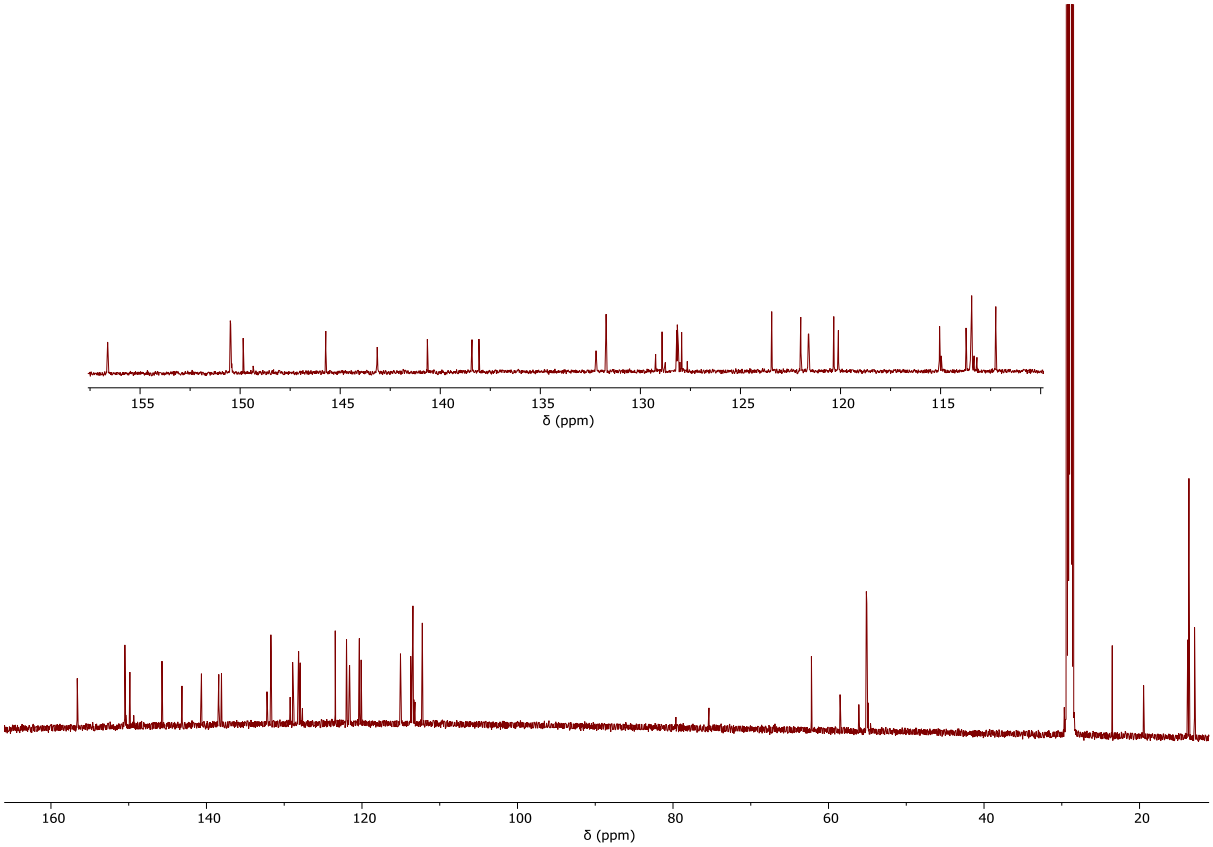


Figure S8. ^{13}C NMR spectrum of **4·HB.**

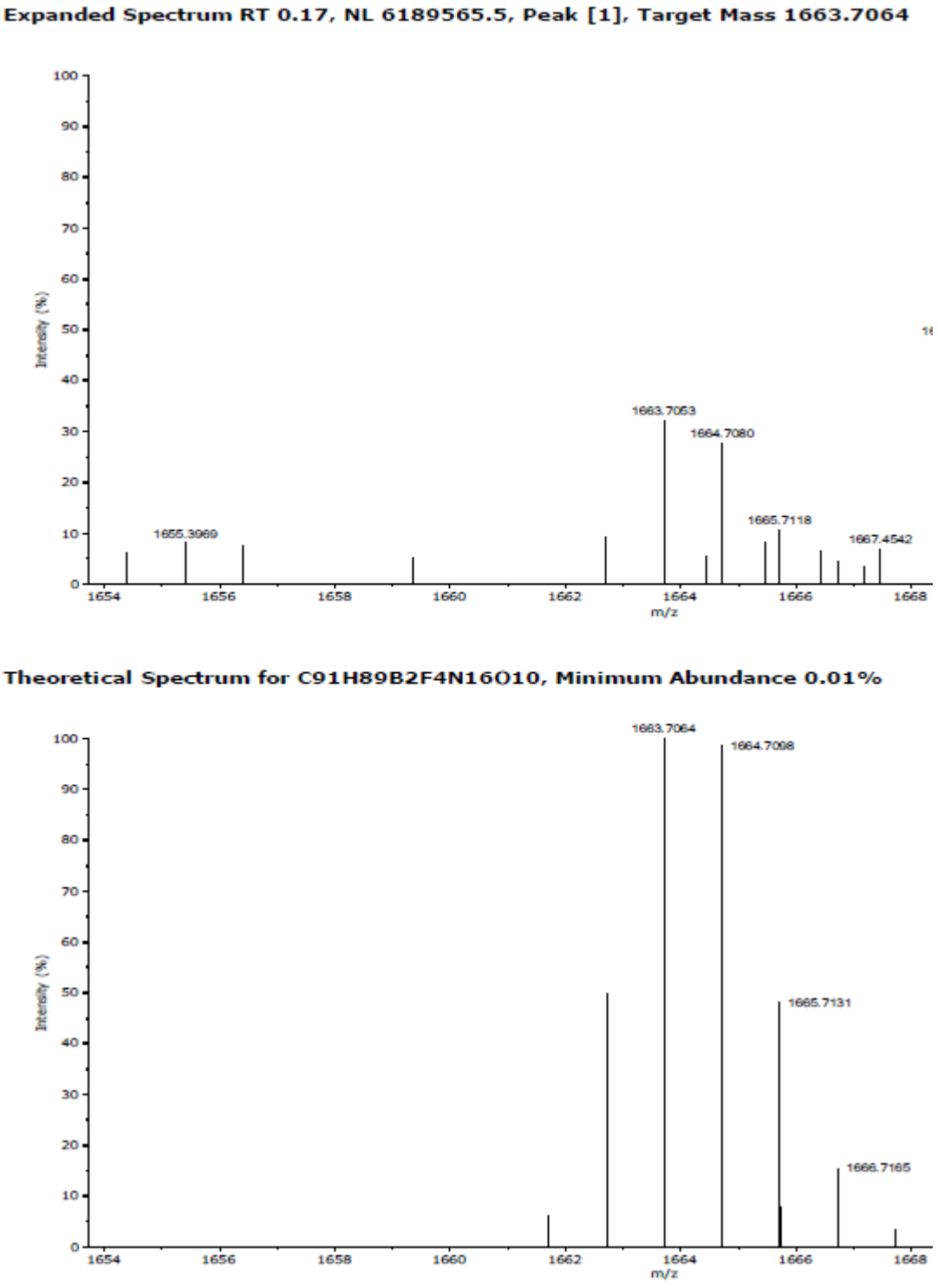


Figure S9. Experimental and theoretical mass spectra of **4·HB.**

3. Optical Characterisation of 4·XB and 4·HB

The absorption and emission spectra for compounds **4·XB** and **4·HB** are shown below. Both receptors showed the expected absorption and emission features for BODIPY-containing compounds, with absorption maxima of 518 nm and 516 nm and emission maxima of 531 nm and 532 nm for **4·XB** and **4·HB** respectively. This corresponds to Stokes shifts of roughly 13-16 nm, which is in line with previous reports.

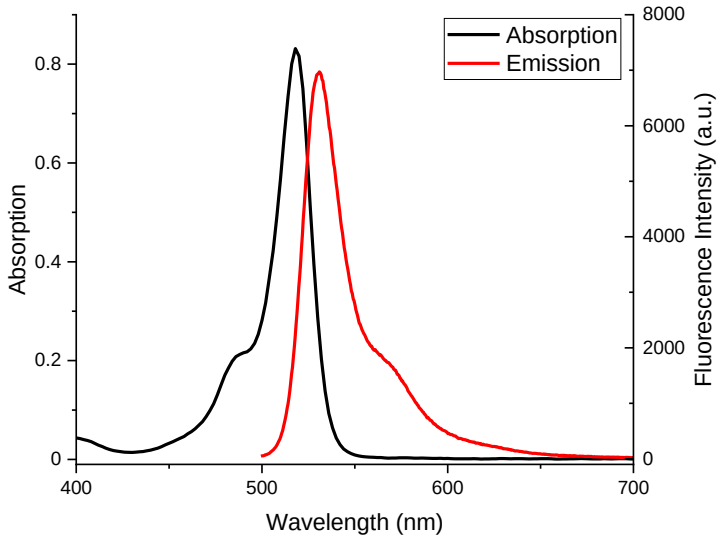


Figure S10. Absorption and emission spectra of **4·XB.** (10 µM, $CHCl_3$, 298K)

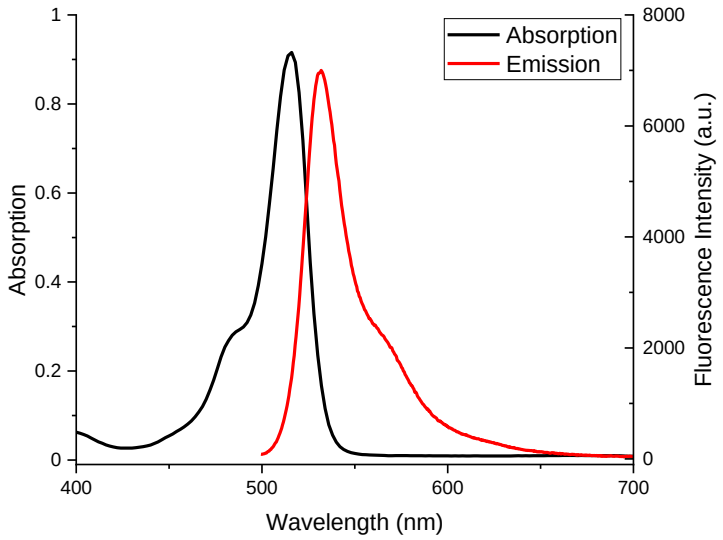


Figure S11. Absorption and emission spectra of **4·HB.** (10 µM, $CHCl_3$, 298K)

4. Fluorescence Titration Studies

As described in the main text, fluorescence titration studies of receptors **4·XB** and **4·HB** with DCB salts were conducted in both $CHCl_3$ and in 50% ACN : H_2O (buffered to pH = 8.0 with 50 mM HEPES); TBAOAc in $CHCl_3$; and with CEES in 50% ACN : H_2O. Representative, stacked emission spectra, with arrows showing the direction of change upon addition of analyte (if any), are shown below for each class of titration. Also shown are emission spectra of **4·XB** and **4·HB** in 50% ACN : H_2O, both before and after addition of thiodiglycol and tributyl phosphate, showing the lack of emission response in each case.[‡]

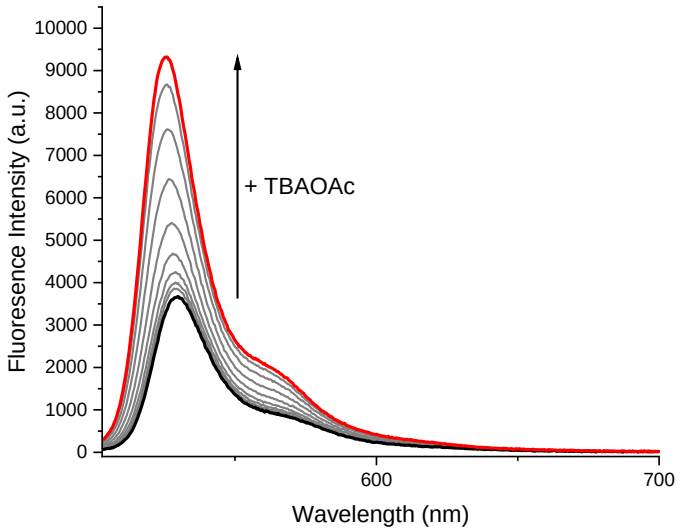


Figure S12. Stacked emission spectra of **4·XB** with increasing concentrations of $^{-}$OAc (up to a maximum of 71 mM), showing the fluorescence response. (1 µM, $CHCl_3$, 298K)

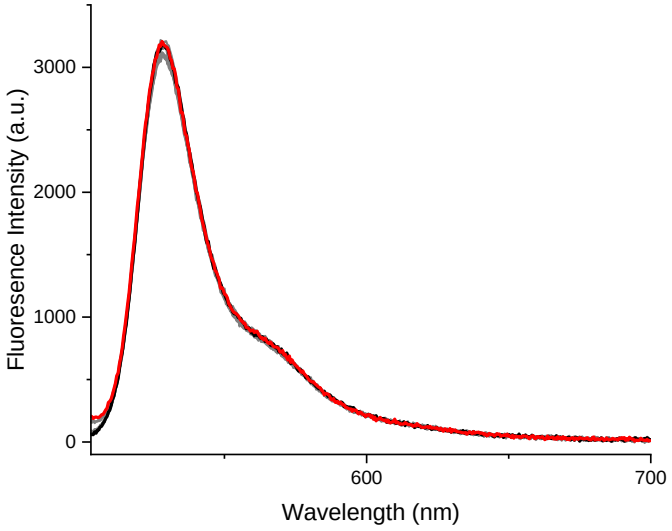


Figure S13. Stacked emission spectra of **4·HB** with increasing concentrations of $^{-}OOC(CH_2)_{10}COO^{-}$ (up to a maximum of 142 µM), showing the lack of a directional fluorescence response. (1 µM, $CHCl_3$, 298K)

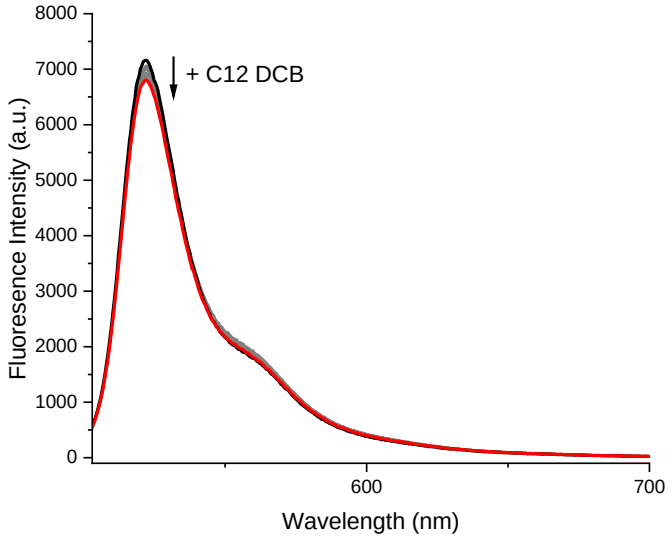


Figure S14. Stacked emission spectra of **4·XB** with increasing concentrations of $^{-}OOC(CH_2)_{10}COO^{-}$ (up to a maximum of 142 µM). (1 µM, 50% ACN : H_2O (buffered to pH = 8.0 with 50 mM HEPES), 298K)

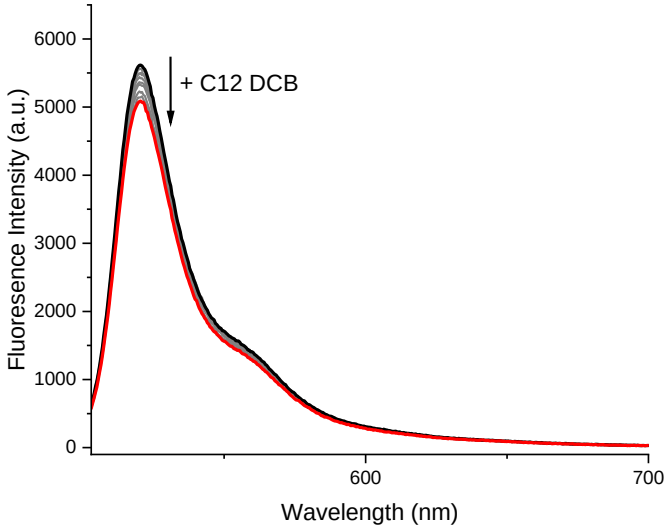


Figure S15. Stacked emission spectra of **4·HB** with increasing concentrations of $^{-}OOC(CH_2)_{10}COO^{-}$ (up to a maximum of 142 µM). (1 µM, 50% ACN : H_2O (buffered to pH = 8.0 with 50 mM HEPES), 298K)

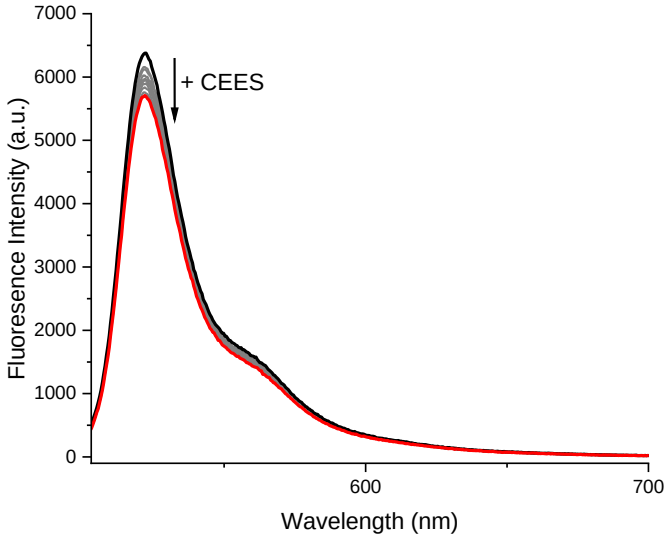


Figure S16. Stacked emission spectra of **4·XB** with increasing concentrations of CEES (up to a maximum of 57 µM). (1 µM, 50% ACN : H_2O, 298K)

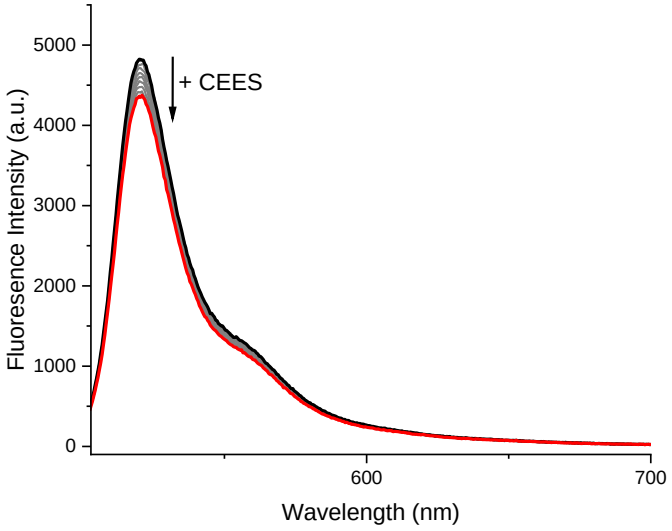


Figure S17. Stacked emission spectra of **4·HB** with increasing concentrations of CEES (up to a maximum of 57 µM). (1 µM, 50% ACN : H_2O, 298K)

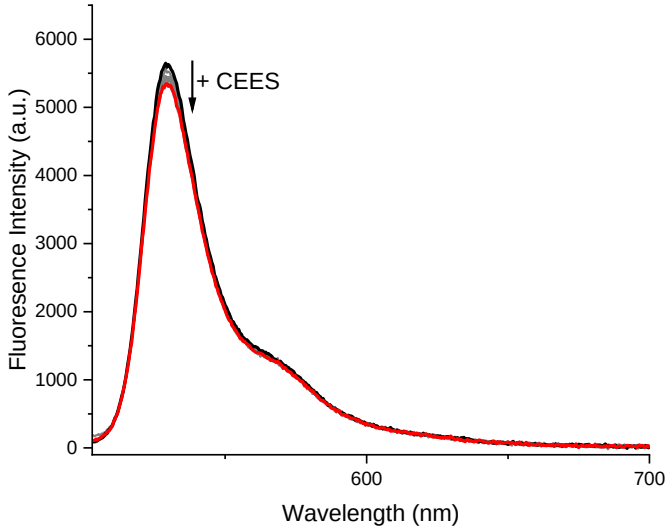


Figure S18. Stacked emission spectra of **4·XB** with increasing concentrations of CEES (up to a maximum of 285 µM). (1 µM, $CHCl_3$, 298K)

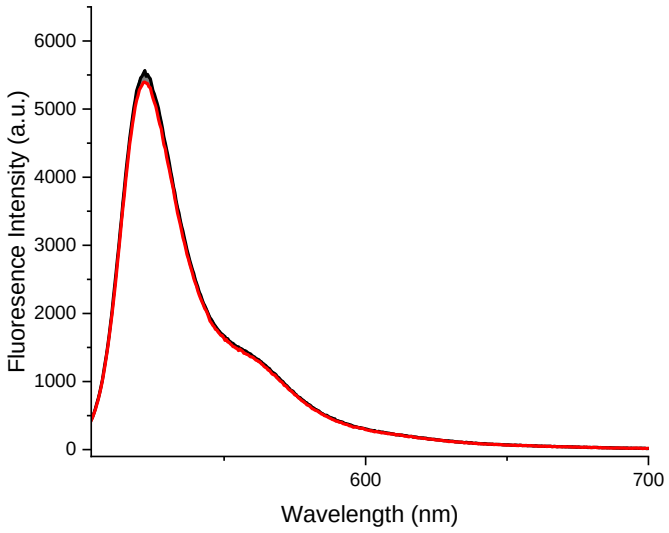


Figure S19. Stacked emission spectra of **4·XB** with increasing concentrations of thiodiglycol (up to a maximum of 57 µM), showing the lack of fluorescence response. (1 µM, 50% ACN : H_2O, 298K)

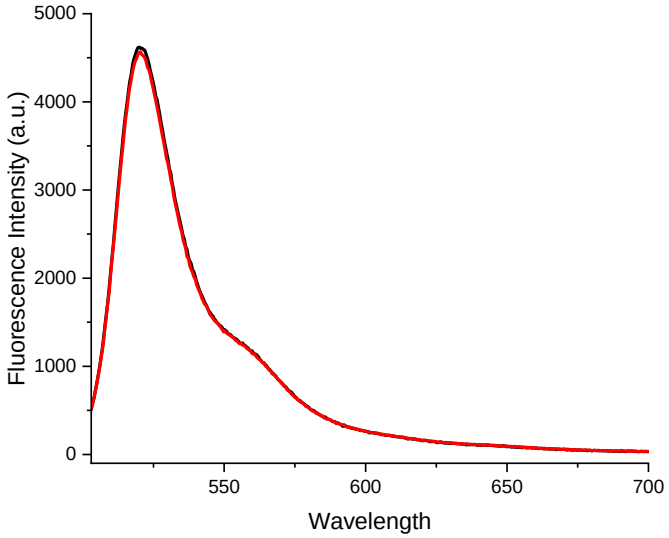


Figure S20. Stacked emission spectra of **4·HB** with and without excess (57 µM) thiodiglycol, showing the lack of fluorescence response. (1 µM, 50% ACN : H_2O, 298K)

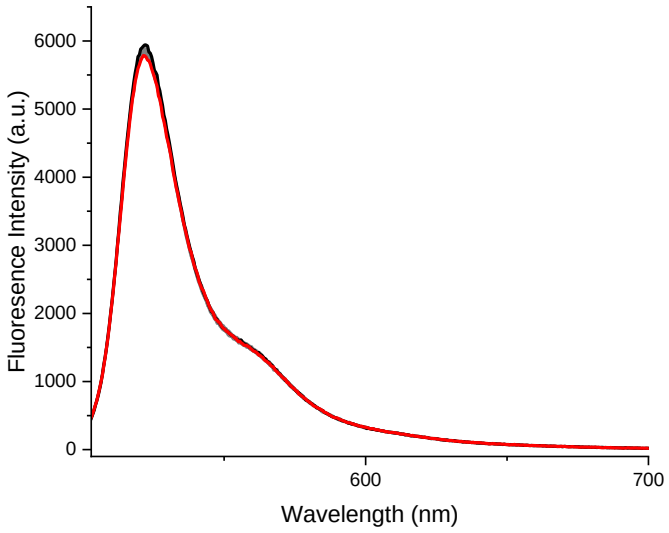


Figure S21. Stacked emission spectra of **4·XB** with increasing concentrations of tributyl phosphate (up to a maximum of 57 µM, showing the lack of fluorescence response. (1 µM, 50% ACN : H_2O, 298K)

5. ^{1}H NMR Threading Studies

To confirm that the DCB analytes thread inside the pillar[5]arene cavity, qualitative ^{1}H NMR studies were conducted in $CDCl_3$, in which aliquots of $(TBA)_2(OOC(CH_2)_{10}COO)$ were added to solutions of the receptors. The stacked spectra for **4·XB** are shown in the main text and the spectra for **4·HB** are presented below. The triazole proton H_d and the P5A phenyl proton H_k both show shifts, indicating threading concurrent with binding to the HB donors.

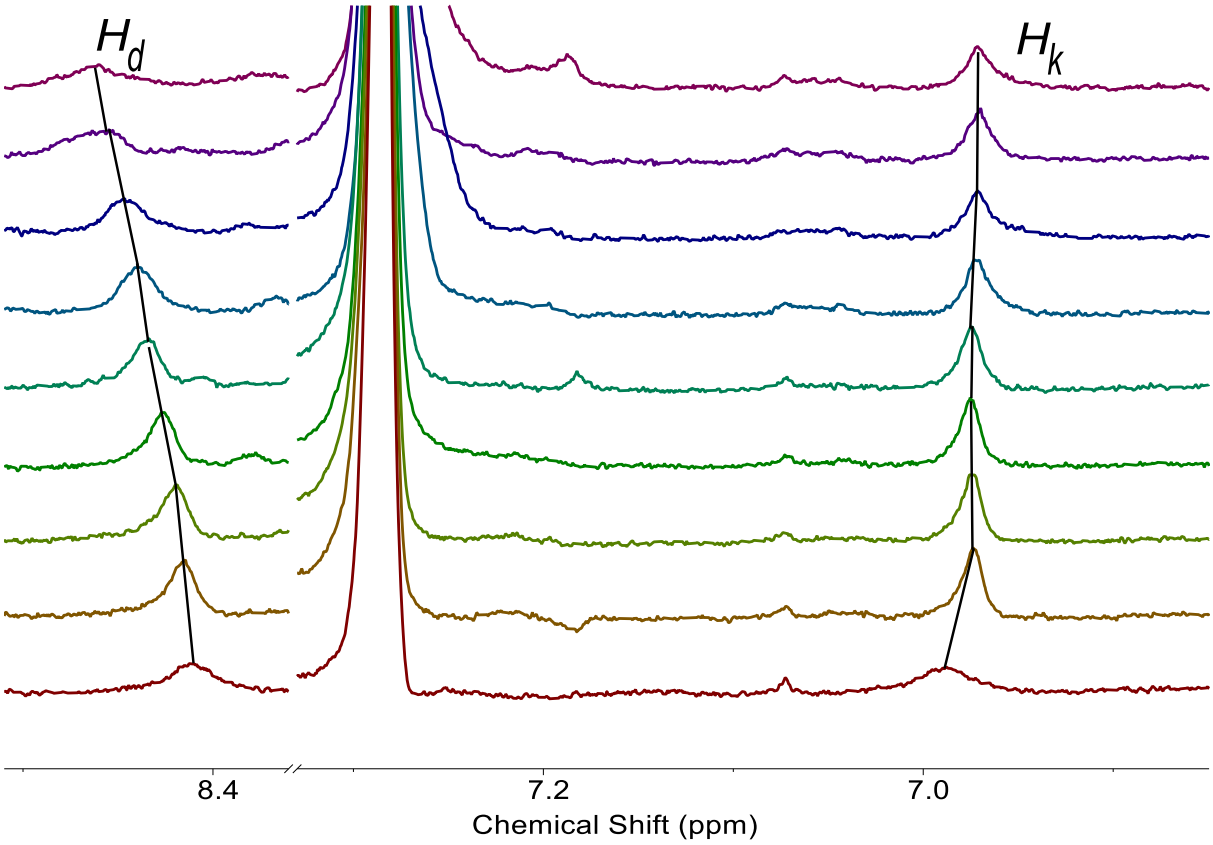


Figure S22. Stacked ^{1}H NMR spectra of **4·HB** upon addition of increasing concentrations of $^-OOC(CH_2)_{10}COO^-$. (1 mM, $CDCl_3$, 298K)

6. Limit of Detection Calculations

In accordance with previous literature reports,[7-8] the limit of detection (LoD) was calculated using the formula:

$$LoD = \frac{3\sigma}{S}$$

σ = Standard deviation of each receptor's emission intensity at the emission maximum.

S = Initial slope of emission intensity curve on addition of analyte, taken from the first four measurements. (M^{-1})

All standard deviations were calculated using three or more emission scans of the receptor (1 µM) before addition of any analyte. The values determined for σ, S and LoD for each receptor are shown in Table S1.

Note that the isotherms shown below are only qualitative. For the determination of binding constants, a global fitting method was used.

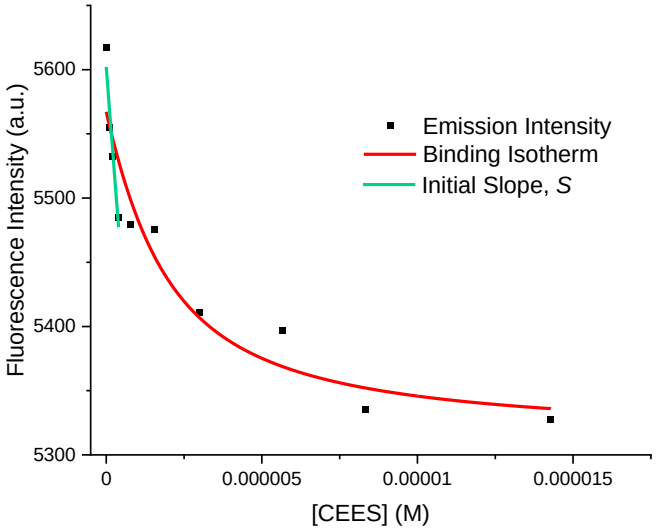


Figure S23. Fluorescence response of **4·XB** at the emission maximum upon addition of increasing concentrations of CEES, the corresponding binding isotherm and the linear line used for the determination of the LoD. (1 µM, 50% ACN : H_2O, 298K)

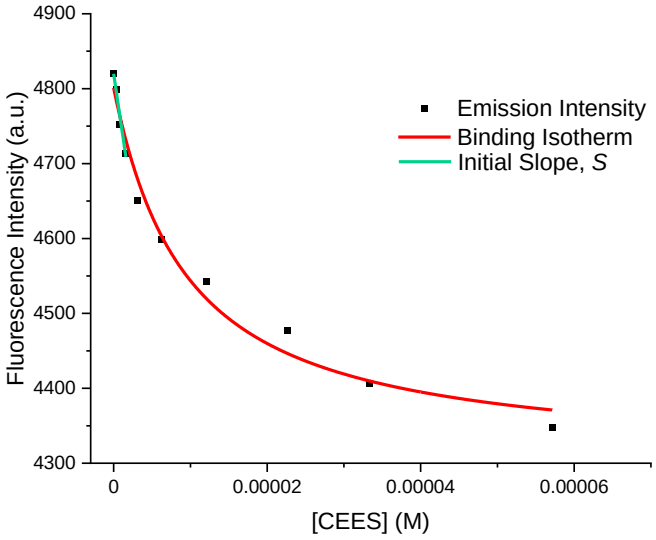


Figure S24. Fluorescence response of **4·HB** at the emission maximum upon addition of increasing concentrations of CEES, the corresponding binding isotherm and the linear line used for the determination of the LoD. (1 µM, 50% ACN : H_2O, 298K)

Receptor	σ	S (M^{-1})	LoD (µM)
4·XB	13.81	3.15×10^8	0.13
4·HB	9.29	6.98×10^7	0.40

Table S1. Calculated values of σ, S and LoD for receptors **4·XB** and **4·HB**.

Notes and References

ǂ A titration of tributyl phosphate with **4·HB** was attempted. However, addition of aliquots of tributyl phosphate to a solution of **4·HB** caused phase separation, preventing the collection of reliable data.

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