Electronic Supplementary Information

Structurally optimised BODIPY derivatives for imaging of mitochondrial dysfunction in cancer and heart cells

Shubhanchi Nigam, Benjamin P. Burke, Laura H. Davies, Juozas Domarkas, Jennifer F. Wallis, Paul G. Waddell, Jennifer Waby, David M. Benoit, Anne-Marie Seymour, Christopher Cawthorne, Lee J. Higham* and Stephen J. Archibald*

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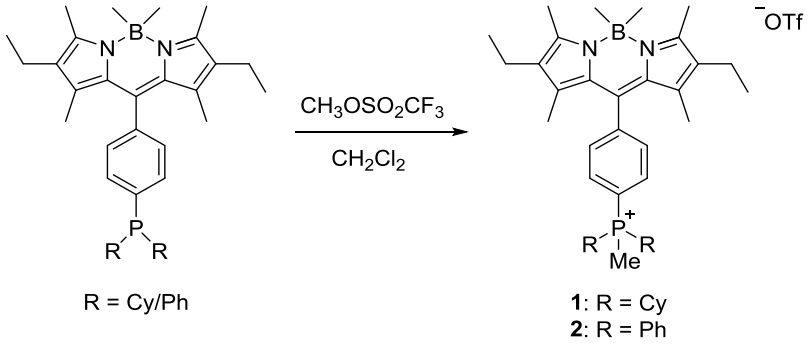
1 Synthetic Details

1.1 General Considerations and Chemical Analysis

All air- and/or water-sensitive reactions were performed under a nitrogen atmosphere using standard Schlenk line techniques. Dichloromethane was dried over calcium hydride and distilled prior to use. Flash chromatography was performed on silica gel purchased from Fluorochem (silica gel, 40-63 μ, 60 Å, LC301). $^{11}B\{^1H\}$, $^{13}C\{^1H\}$, ^1H and $^{31}P\{^1H\}$ NMR spectra were recorded on a JEOL ECS-400 (^1H 399.78 MHz) spectrometer at room temperature (21 °C) using the indicated solvent as internal reference. Mass spectrometry was carried out by the EPSRC National Mass Spectrometry Service Centre, Swansea. Thin layer chromatography was performed on Merck aluminium-based plates with silica gel and fluorescent indicator (254 nm).

1.2 General Synthetic Scheme to **1** and **2**



Scheme S1. Synthesis of **1** and **2**.

1.3 Synthesis of **1**

8-((4-dicyclohexylphosphino)phenyl)-4,4-dimethyl-1,3,5,7-tetramethyl-2,6-diethyl-4-bora-3a,4a-diaza-*s*-indacene (0.21 g, 0.36 mmol) was dissolved in dichloromethane (4 mL) and methyl trifluoromethanesulfonate (0.08 mL, 0.73 mmol) was added. The reaction mixture was stirred at room temperature for two hours, which produced an orange precipitate. The solid was filtered and washed with petroleum ether (3 × 20 mL) to give the intended product as a fine orange solid (0.18 g, 68%). **^1H NMR** (400 MHz, CD_3CN) δ 7.82 (dd, $^3J_{HH}$ = 8.2 Hz, $^3J_{HP}$ = 11.0 Hz, 2H), 7.67 (dd, $^3J_{HH}$ = 8.2 Hz, $^4J_{HP}$ = 2.6 Hz, 2H), 2.71 (m, 2H), 2.43 (s, 6H), 2.31 (q, $^3J_{HH}$ = 8.2 Hz, 4H), 2.04 (d, $^2J_{HP}$ = 12.4 Hz, 3H), 1.98-1.69 (br, 10H), 1.40 (m, 4H), 1.26-1.07 (br, 6H), 1.20 (s, 6H), 0.93 (t, $^3J_{HH}$ = 8.2 Hz, 6H), 0.30 (s, 6H) ppm; **$^{31}P\{^1H\}$ NMR** (162 MHz, CD_3CN) δ 34.5 ppm; **$^{11}B\{^1H\}$ NMR** (128 MHz, CD_3CN) δ −1.6 ppm. Low solubility precluded the collection of a ^{13}C NMR spectrum. **HRMS** (ESI^+) exact mass calculated for $C_{38}H_{57}N_2B_1P_1$ $[M]^+$ requires m/z 582.4383, found *m/z* 582.4382 (0.2 ppm).

1.4 Synthesis of **2**

8-((4-diphenylphosphino)phenyl)-4,4-dimethyl-1,3,5,7-tetramethyl-2,6-diethyl-4-bora-3a,4a-diaza-*s*-indacene (0.13 g, 0.22 mmol) was dissolved in dichloromethane (4 mL) and methyl trifluoromethanesulfonate (0.07 mL, 0.45 mmol) was added. The reaction mixture was stirred at room temperature for two hours. After removal of the solvent, purification was performed by column chromatography (chloroform/methanol, 10:0.3, R_f = 0.3) on silica gel to yield the intended product as an orange solid (0.35 g, 21%). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.75-7.70 (m, 4H), 7.66-7.59 (m, 10H), 3.01 (d, $^2J_{HP}$ = 13.7 Hz, 3H), 2.38 (s, 6H), 2.23 (q, $^3J_{HH}$ = 7.8 Hz, 4H), 1.19 (s, 6H), 0.91 (t, $^3J_{HH}$ = 7.8 Hz, 6H), 0.20 (s, 6H) ppm; **^{13}C{^{1}H} NMR** (100 MHz, $CDCl_3$) δ 151.8, 145.1 (d, J_{CP} = 2.8 Hz), 136.6, 135.3 (d, J_{CP} = 2.9 Hz), 133.4 (d, J_{CP} = 11.5 Hz), 133.1 (d, J_{CP} = 11.5 Hz), 133.0 (d, J_{CP} = 39.3 Hz), 131.3 (d, J_{CP} = 13.4 Hz), 130.6 (d, J_{CP} = 13.4 Hz), 128.2, 119.8, 119.2, 118.3, 117.7 (q, J_{CF} = 249.2 Hz), 17.4, 14.6, 14.4, 12.1, 10.4 (br), 9.34 (d, J_{CP} = 57.5 Hz) ppm; **^{31}P{^{1}H} NMR** (162 MHz, $CDCl_3$) δ 22.6 ppm; **^{11}B{^{1}H} NMR** (128 MHz, $CDCl_3$) δ −1.9 ppm; **HRMS** (ESI^+) exact mass calculated for $C_{38}H_{45}N_2B_1P_1$ $[M]^+$ requires m/z 570.3444, found *m/z* 570.3443 (0.2 ppm).

1.5 ^{1}H NMR Spectrum of **1**

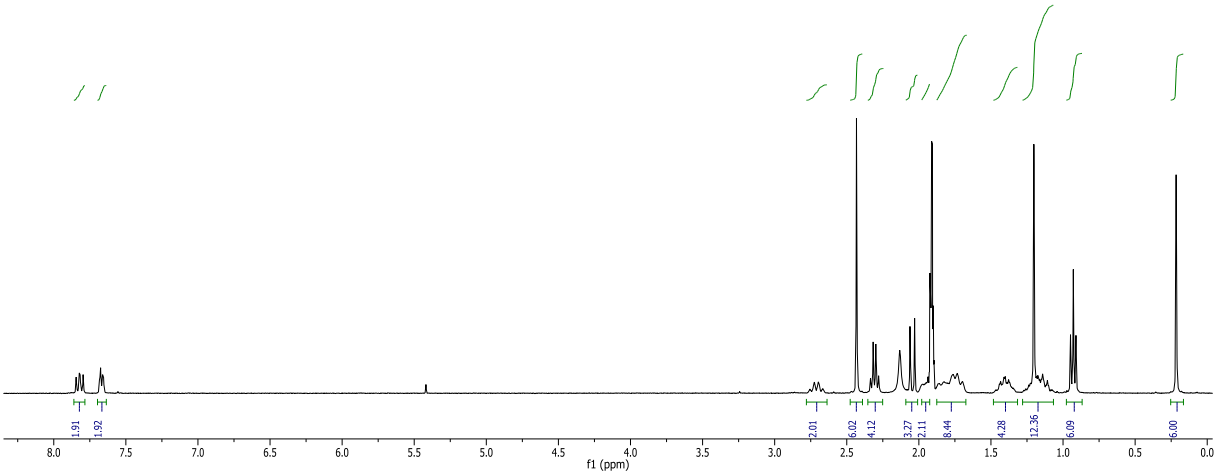


Figure S1. ^{1}H NMR Spectrum of **1**.

1.6 ^{1}H NMR Spectrum of **2**

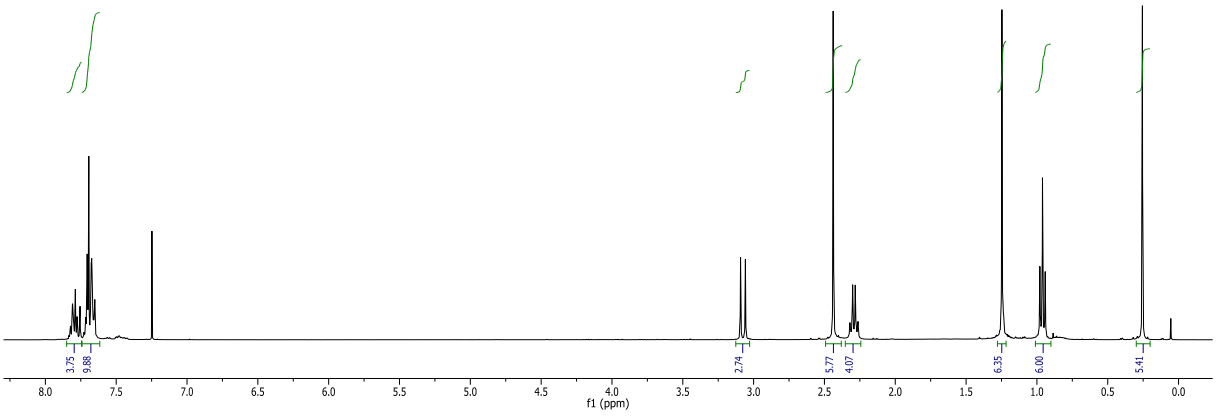
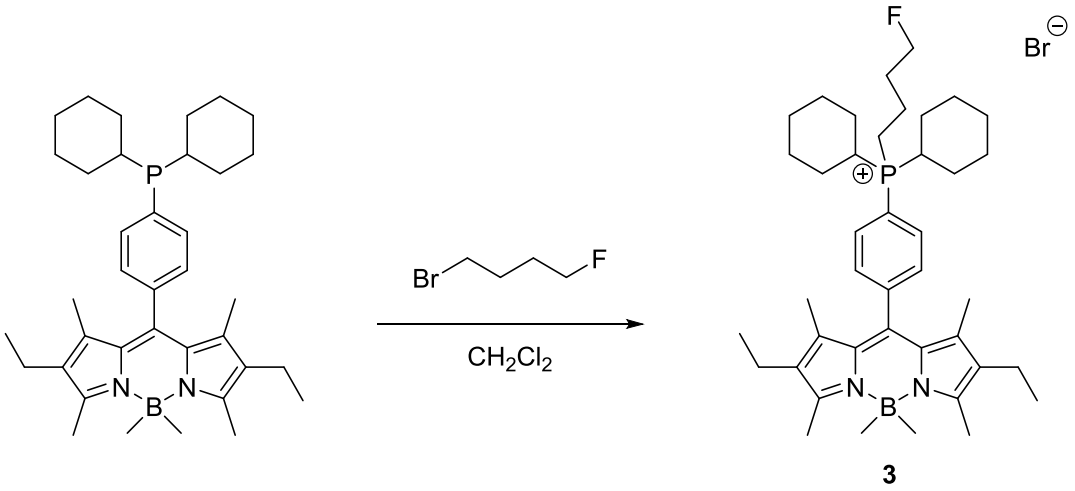


Figure S2. ^{1}H NMR Spectrum of **2**.

1.7 Synthesis of **3**



3

Scheme S2. Synthesis of **3**.

8-((4-dicyclohexylphosphino)phenyl)-4,4-dimethyl-1,3,5,7-tetramethyl-2,6-diethyl-4-bora-3a,4a-diaza-s-indacene (0.066 g, 0.12 mmol) was dissolved in anhydrous DCM (2 mL), to the flask was added 1-bromo-4-fluorobutane (0.012 mL, 0.12 mmol). The mixture was heated to 40 °C for 48 hours. The solvent was removed and the product was crystallised from deuterated chloroform / pentane to give the product as dark orange crystals. **^{1}H NMR** (300 MHz, $CDCl_3$) δ 8.09 (m, 2H), 7.70 (m, 2H), 4.65 (t, *J* = 5.2 Hz, 1H), 4.50 (t, *J* = 5.2 Hz, 1H), 3.20 (m 4H), 2.44 (s, 6H), 2.28 (q, *J* = 7.6 Hz, 4H), 2.22-1.73 (m, 16H), 1.58-1.32 (m, 10H), 1.17 (s, 6H), 0.96 (t, *J* = 7.5 Hz, 6H), 0.26 (s, 6H) ppm; **^{13}C{^{1}H} NMR** (176 MHz, $CDCl_3$) δ 151.8, 144.4, 136.9, 133.4 (d, J_{CP} = 7.5 Hz), 133.4, 132.8, 131.3 (d, J_{CP} = 11.4 Hz), 128.4, 83.7, 82.8, 31.2 (m), 30.4 (d, J_{CP} = 43.5 Hz), 26.6 (dd, J_{CP} = 13.0 Hz, J_{CP} = 2.9 Hz), 26.3 (m), 25.6, 19.5, 17.5, 15.9 (d, J_{CP} = 44.4 Hz), 14.8, 14.5, 12.1, 10.4 (br) ppm; **^{31}P NMR** (121 MHz, $CDCl_3$) δ 32.6 ppm; **^{19}F{^{1}H} NMR** (470 MHz, $CDCl_3$) δ – 221. 3 ppm, **^{11}B NMR** (96 MHz, $CDCl_3$) δ – 0.4 ppm.

1.8 NMR analysis of **3**

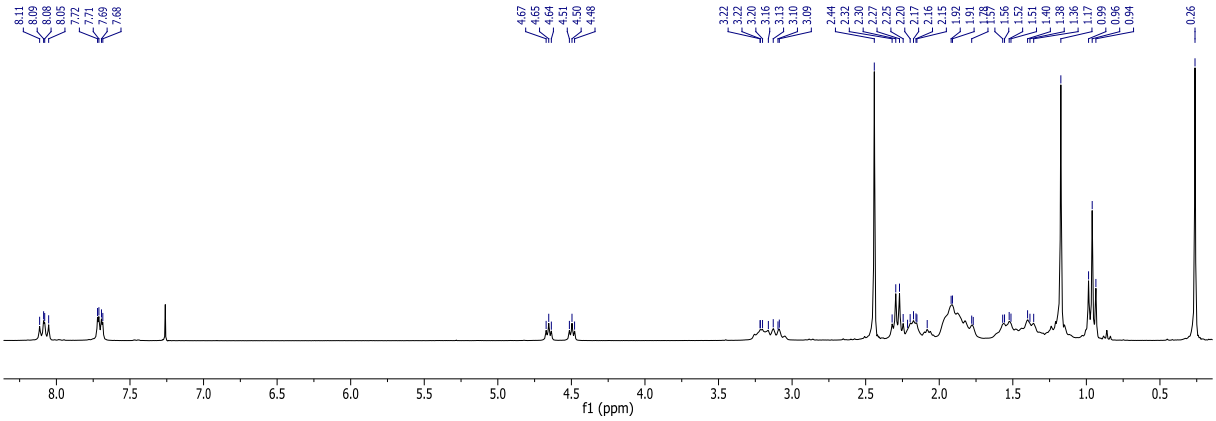


Figure S3. ^{1}H NMR Spectrum of **3**.

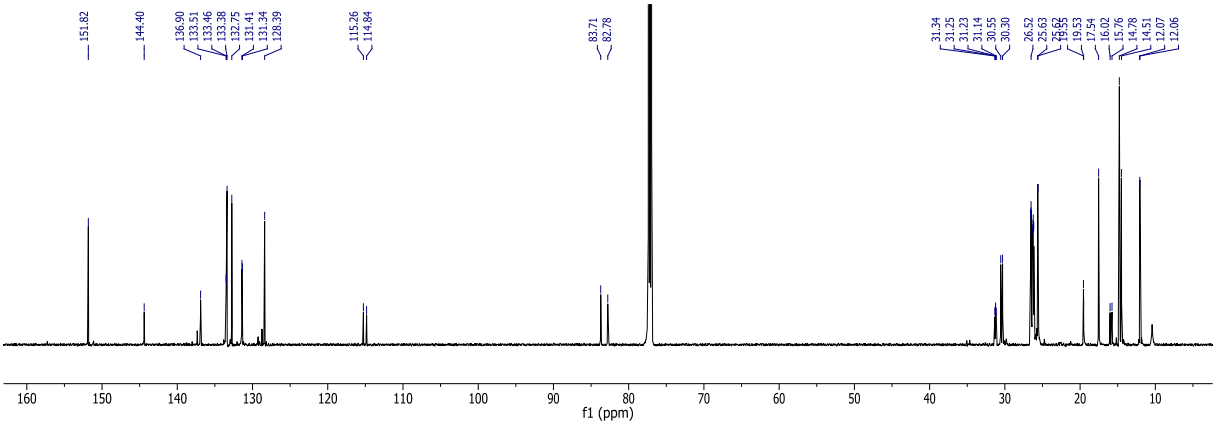


Figure S4. ^{13}C NMR Spectrum of **3**.

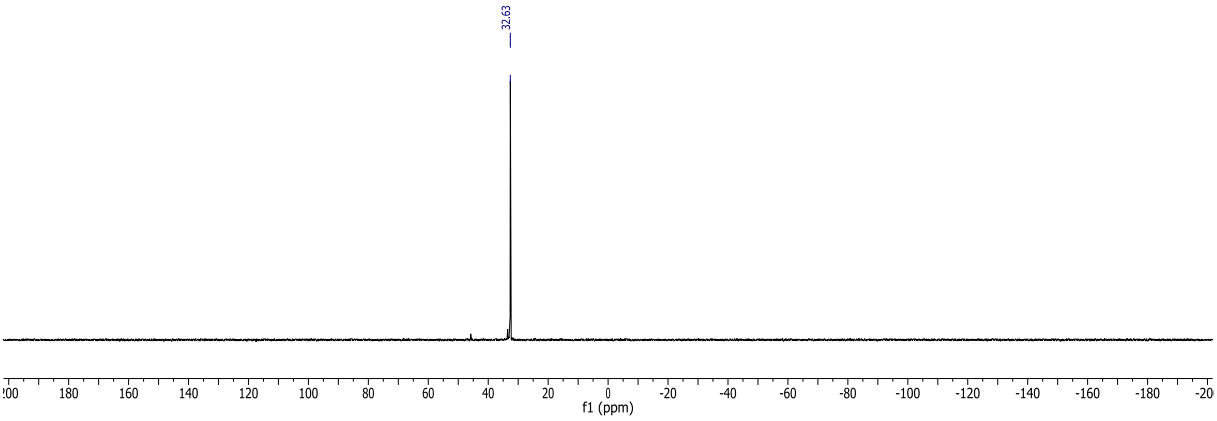


Figure S5. ^{31}P NMR Spectrum of **3**.

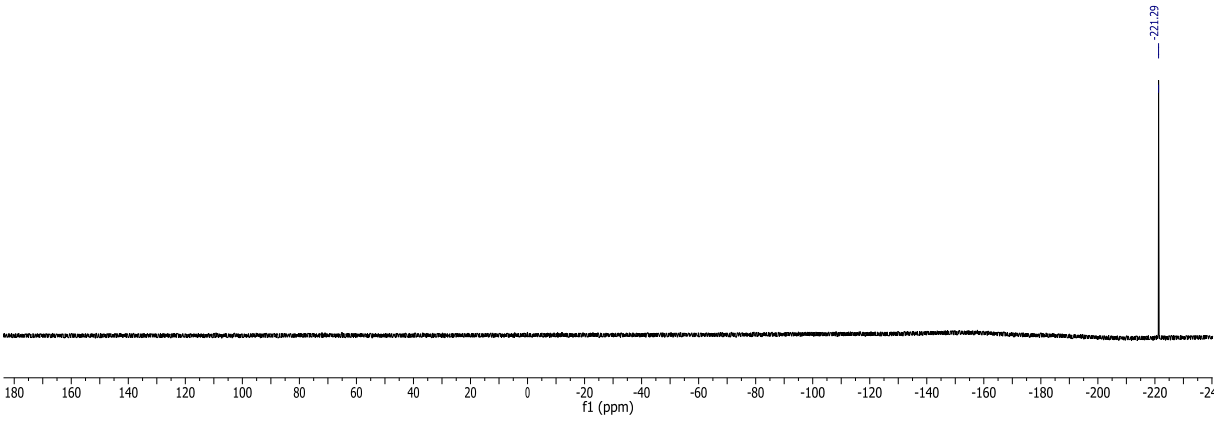


Figure S6. $^{19}F\{1H\}$ NMR Spectrum of **3**.

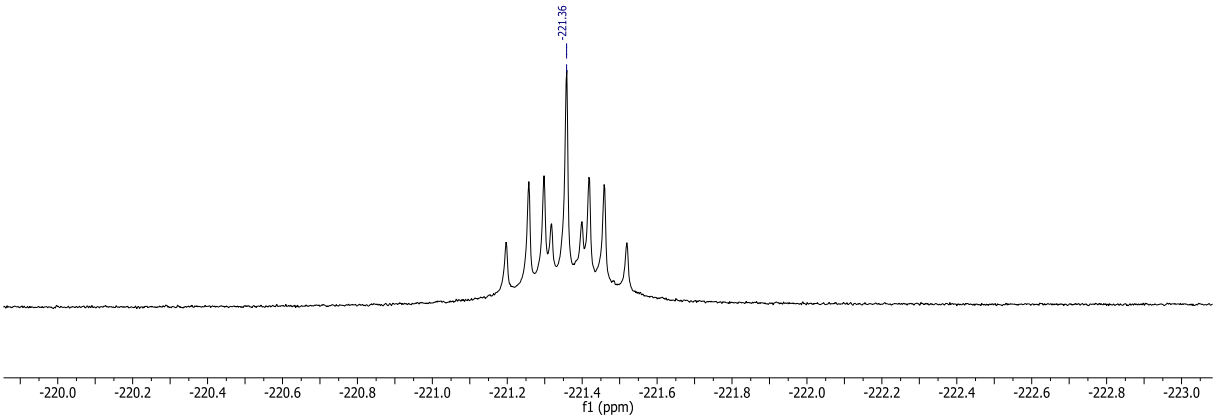


Figure S7. ^{19}F NMR Spectrum of **3**.

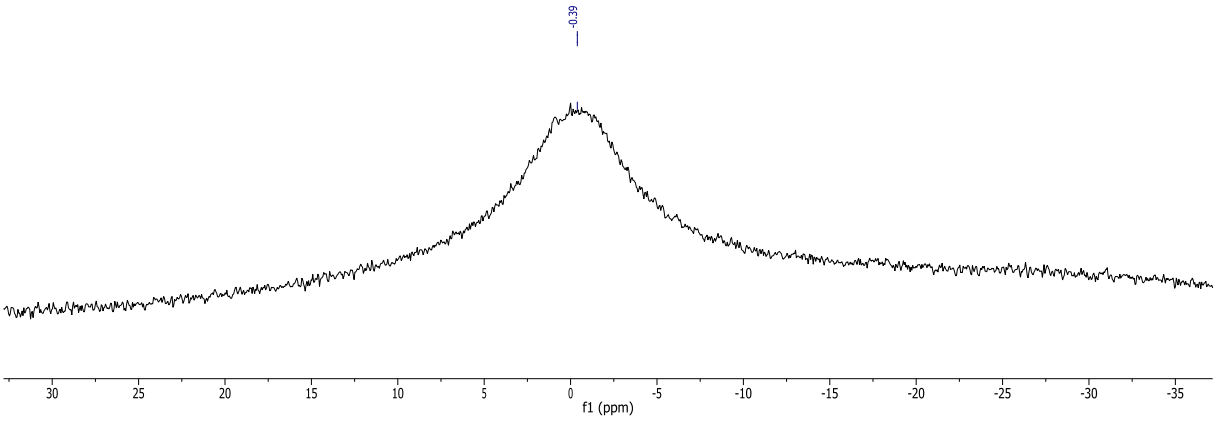


Figure S8. ^{11}B NMR Spectrum of **3**.

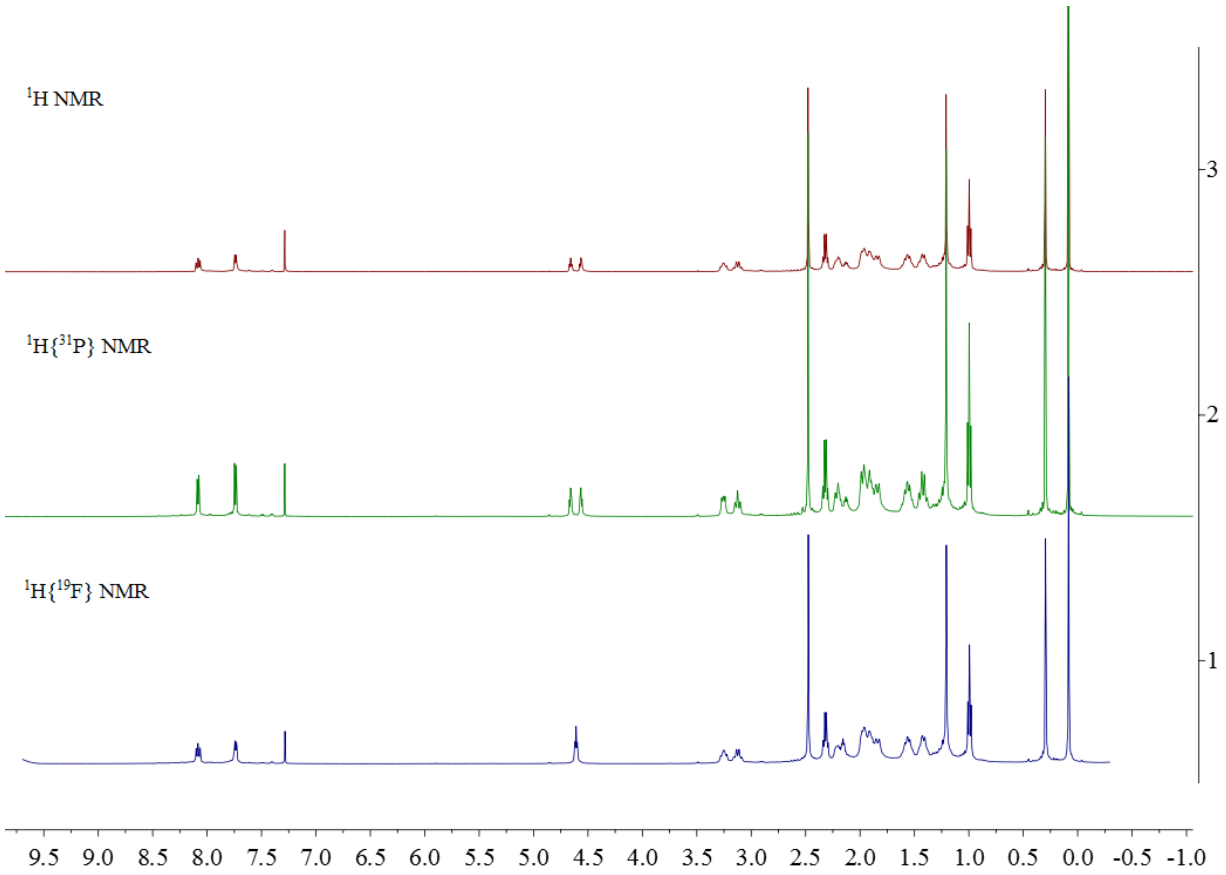


Figure S9. Overlapped ^{1}H NMR Spectrums of **3** with P and F decoupling.

2 Excitation and Emission Spectra of 1 and 2

Absorption spectra were recorded with a Hitachi Model U-3310 spectrophotometer while fluorescence studies were recorded with a Hitachi F-4500 fluorescence spectrophotometer. Absorption and emission spectroscopy were recorded for all compounds in dry degassed tetrahydrofuran, at room temperature. **1** and **2** were excited at 485 nm and excitation and emission slits were both set to 5 nm.

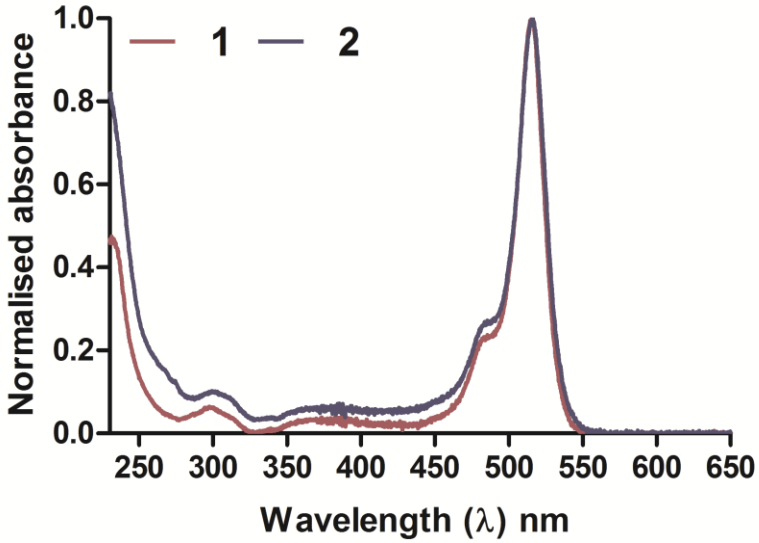


Figure S10. Absorption spectra of **1** and **2**.

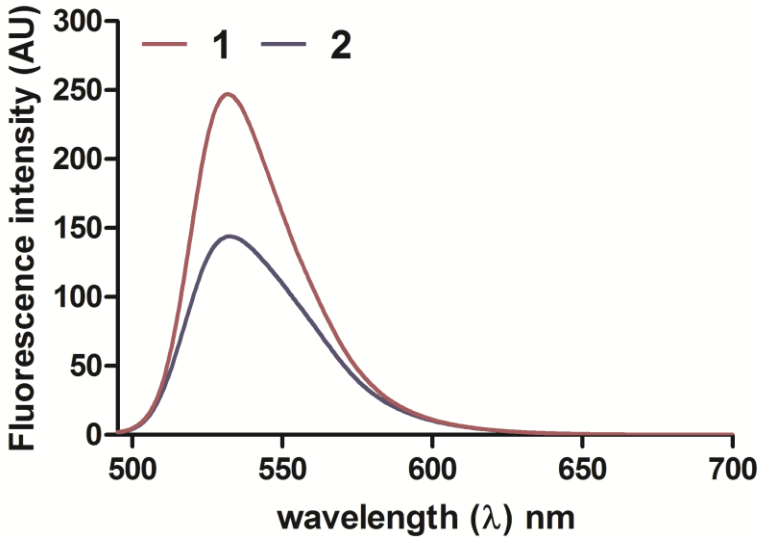


Figure S11. Emission spectra of **1** and **2** after excitation at 485 nm.

3 Partition-coefficient (LogP) Determination

The lipophilicity of **1** and **2** was measured using logP values. Products **1** and **2** were dissolved in octanol and phosphate-buffered saline (PBS) at 7.4 pH over a concentration range, and the fluorescence of the different samples was measured. Standard curves of fluorescence intensity *vs* concentration were plotted in order to assess the optimum concentration for partition-coefficient measurements. To a mixture of PBS (500 µL) and octanol (500 µL) in a microcentrifuge tube was added, respectively, a stock solution of **1** or **2** (500 nM, 50 µL). The contents were vigorously mixed by vortex mixer for 30 min and then the phases were separated by centrifugation (2000 x *g* for 10 min). Octanol and PBS phases were carefully dispensed onto multiwell plates and fluorescence measured with a Tecan Infinite instrument (Tecan, Reading, UK). Each experiment was carried out independently in triplicate, along with three internal triplicates. LogP was determined using the following equation:

$$LogP = \mathrm{Log}\frac{[\mathrm{Octanol}]}{[\mathrm{PBS}]}$$

1 = LogP = 3.19 ± 0.13

2 = LogP = 2.80 ± 0.08

3.1 Fluorescence Intensity *vs* Concentration Calibration Curve Determination for **1**

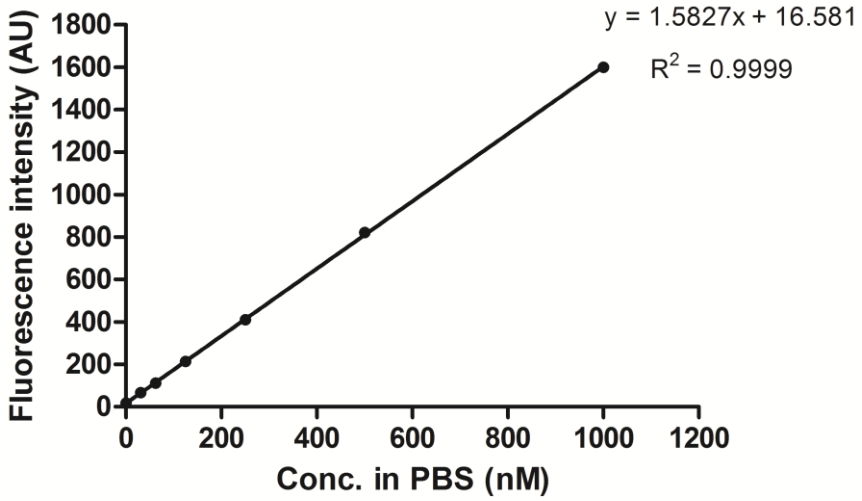


Figure S12. Calibration curve of **1** in PBS.

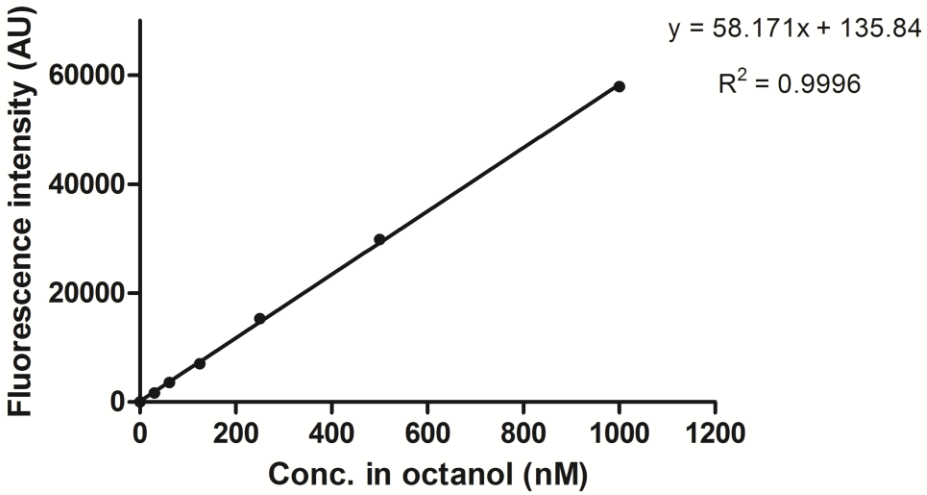


Figure S13. Calibration curve of **1** in octanol.

3.3 Fluorescence Intensity vs Concentration Calibration Curve Determination for **2**

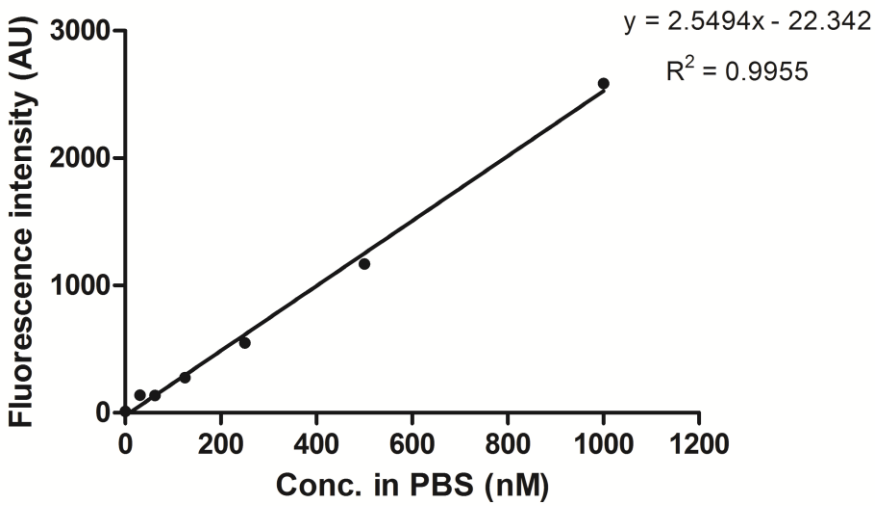


Figure S14. Calibration curve of **2** in PBS.

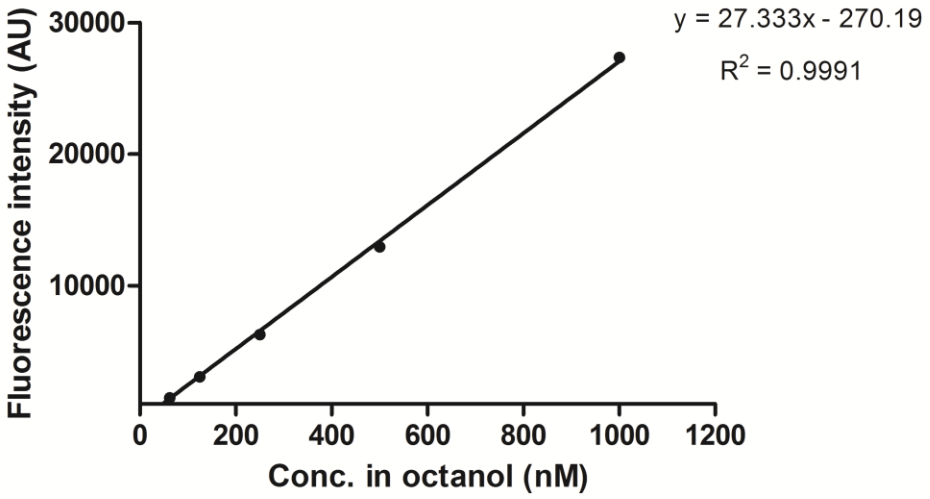


Figure S15. Calibration curve of **2** in octanol.

4 Cell Culture

MCF-7 cells were purchased from the European Collection of Cell Cultures and grown in PRMI-1640 media. H9c2 (2-1 clone) cells were purchased from LGC-PromoChem (Teddington, UK) and grown in IMDM media. Media was supplemented with 10% fetal calf serum (FCS). Media and FCS were purchased from Gibco/Life Technologies, UK.

Cells were maintained in Nunc 75 cm^2 tissue culture flasks (Fischer Scientific, UK) inside a humidified 5% CO_2 incubator at 37 ^{0}C. Both cell lines were divided bi-weekly for maintenance of stock at a divided ratio of 1:8-1:10 for MCF-7 cells and 1:3-1:5 for H9c2 cells. For confocal and flow cytometry experiments cells were counted and seeding densities (below) were used.

5 Confocal Microscopy

5.1 Experimental Details

MCF-7 cells (2.5 x 10^3) and H9c2 cells (4 x 10^3) were seeded in Mattak confocal dishes (P35G-1.2-20-C, 35 mm^2) and cultured for 2-3 days until 70-80% confluent. After reaching desired cell density, the growth media was replaced with 1 mL 0.1% FCS-containing media and cells were treated with compound **1** or **2** (500 nM, 50 µL) for 1 hour inside a CO_2 incubator under 5% CO_2. Mitotracker deep red (MDR, 250 nM, 20 µL) was added 1 hour prior to compound to obtain double labelled cells for mitochondrial co-localisation study. After 30 min, the cells were washed twice with 1 mL PBS and media containing 15 mM HEPES was added. Unstained control samples of cells were used to check for auto fluorescence.

Live cell images were obtained using ZEN software on a Zeiss LSM 710 inverted confocal microscope, equipped with an incubator chamber at 37 °C. Images were obtained using a Zeiss 63x water objective.

5.2 Colocalisation of **1** and **2** with Mitotracker Deep Red (MDR)

5.2.1 Method for Calculating Colocalisation

To measure the degree of overlap between subcellular localisation of **1** or **2** and MDR, a colocalisation coefficient was calculated following standard methods.[1] Single-label control samples were prepared to eliminate fluorescence overlap between two fluorescence channels; in addition to **1**, **2** or MDR, untreated cells were assessed in order to eliminate background fluorescence. Single-label control samples were imaged under the same exposure settings as double-labelled samples. Exact coordinates were determined using crosshairs at X and Y coordinates from single-labelled controls and were identical for double-labelled samples. Once the exact coordinates were determined, the ZEN software gave tables containing values of co-localisation coefficient and overlap coefficient.

5.2.2 Colocalisation Tables

Cell line	Colocalisation coefficient		Overlap Coefficient
	1	MDR	
MCF-7	0.77	0.66	0.70
H9c2	0.60	0.69	0.71

Cell line	Colocalisation coefficient		Overlap Coefficient
	2	MDR	
MCF-7	0.62	0.53	0.52
H9c2	0.62	0.55	0.69

6 Flow Cytometry

1 x 10^5 cells/well were seeded in six-well plates and cultured for 2-3 days until 70-80% confluent. Cell media was removed and 1 mL 0.1% FCS-containing media was added. Carbonyl cyanide *m*-chlorophenylhydrazone (CCCP, Sigma Aldrich, UK) in DMSO (50 mM, 1 μL) was added into three wells (1 mL total volume) and 0.1% DMSO was added to the remaining three control wells (as a control). Plates were placed inside a CO_2 incubator for 30 mins, followed by the addition of samples of **1**, **2** or **3** (500 nM, 50 μL media) to both groups. Plates were incubated inside the CO_2 incubator at 37 ^{0}C for 1 hour, subsequently, media was removed and the cells were washed twice with ice cold PBS buffer. Cells were harvested into PBS with cell scraper and isolated by centrifugation at 200 x *g* for 5 min at 4 °C. The pellet was re-suspended in 300 μL of PBS and the cell suspension transferred into polypropylene FACS tubes (Falcon 2054) and analysed by a FACScan flow cytometer (BD Biosciences Europe, Erembodegem,

Belgium). Dot plot was used to gate cells and the FL-1 channel was used to measure variation in fluorescence intensities. Mean fluorescence intensities were used to quantify for comparison.

6.1 Mean Fluorescence Intensity (MFI)

Cell line	Mean Fluorescence Intensity of **1**		Percentage Decrease (%)
	-CCCP	+CCCP	
MCF-7	3301	968	70.7
H9c2	5197	1472	71.7

Cell line	Mean Fluorescence Intensity of **2**		Percentage Decrease (%)
	-CCCP	+CCCP	
MCF-7	641	400	37.6
H9c2	3589	1520	57.6

Cell line	Mean Fluorescence Intensity of **3**		Percentage Decrease (%)
	-CCCP	+CCCP	
MCF-7	2269	373	83.6
H9c2	1928	321	83.3

7 Computational Chemistry

The geometries of both BODIPY structures (**1** and **2**) were fully optimised without $^{-}$OTf counter ions using density functional theory, the TPSS exchange–correlation functional[2] and a def2-TZVPP basis set,[3] as implemented in the ORCA 3.0.3 suite of programs.[4] We used the resolutions of the identity (RI) approximation and a def2-TZVPP/J auxiliary basis set[5] to accelerate the calculations. In order to account for dispersion interactions, which are often severely underestimated in density functional approaches, we use Grimme's D3 empirical corrections[6] with Becke–Johnson damping[7] for all calculations (TPSS-D3 method). The atomic charges are obtained for the minimum energy structures through a CHELPG fitting procedure[8] and are constrained to reproduce the electrostatic potential around the molecule while conserving the total charge of the molecule. The computed charges along with the atomic positions of the optimised structures are shown below.

7.1 Table of optimised atomic positions and CHELPG charges for structure **1**.

	X/Å	Y/Å	Z/Å	Charge/e
N	2.257649507	-4.919417225	2.700888202	-0.293231
C	2.459134788	-6.252544307	2.31590029	-0.318506
C	2.449245557	-6.602103695	0.96393105	0.2637
C	2.221870336	-5.681394913	-0.063618778	-0.291862
N	1.992438595	-4.332489386	0.239667036	-0.28928
B	2.077756542	-3.686192971	1.699865224	1.128876
C	2.307613916	-4.872447173	4.047956687	0.423034
C	2.529827166	-6.179270286	4.574786741	-0.371072
C	2.629036956	-7.051505749	3.496657366	0.267889
C	2.156292513	-5.849738586	-1.487637797	0.200521
C	1.881452559	-4.592478878	-2.017213185	-0.385711
C	1.786047005	-3.680869957	-0.923869389	0.455266
C	3.438882712	-2.791409521	1.767253265	-0.78998
C	0.671629047	-2.944353867	2.02898337	-0.82342
C	2.135654205	-3.639594253	4.871561388	-0.523884
C	2.66803601	-6.501892536	6.034179251	0.191615
C	4.127555453	-6.435726555	6.523726852	-0.300595
C	2.864188742	-8.529321351	3.609318176	-0.367085
C	2.329012573	-7.099940864	-2.300381511	-0.293395
C	1.668469385	-4.23035609	-3.457316153	0.236347
C	0.186244939	-4.277398741	-3.875043069	-0.294116
C	1.489725887	-2.223819302	-1.045636505	-0.613234
C	2.681462851	-8.02335423	0.595244766	0.041585
C	1.598343692	-8.882085455	0.367624537	0.089004
C	1.811217921	-10.20329013	-0.001850508	-0.372864
C	3.121206887	-10.69168919	-0.152103321	0.209246
C	4.2065038	-9.835687595	0.082914995	-0.306014
C	3.983250149	-8.514538219	0.458246473	0.017816
P	3.36489216	-12.3812025	-0.703199597	0.449309
C	2.368680322	-13.43200966	0.429438387	0.002806
C	2.665221523	-12.59840453	-2.399198992	0.119799
C	5.140287786	-12.72609047	-0.62488792	-0.619731
C	3.336287348	-13.73494803	-3.215087169	-0.073502
C	4.639195229	-13.32154952	-3.917816041	0.00243
C	4.492705176	-12.01308057	-4.703651219	-0.102306
C	3.948709994	-10.89245416	-3.810903463	0.08229
C	2.59566628	-11.28963223	-3.216245054	-0.23299
C	2.839145325	-13.35623344	1.902216009	-0.12378
C	3.962517653	-14.34506033	2.247779915	0.019055
C	3.628002875	-15.77326693	1.801939044	-0.116749
C	3.302458724	-15.82289551	0.304514522	0.034227
C	2.135332661	-14.88771422	-0.029272106	-0.080496

H	3.615179657	-2.357226956	2.757931874	0.135635
H	3.434181082	-1.950974733	1.064031716	0.133828
H	4.320625065	-3.400498842	1.526920728	0.119999
H	-0.176351668	-3.636201336	1.934381282	0.116943
H	0.471637439	-2.100800359	1.361155323	0.142478
H	0.636506005	-2.535318199	3.043344648	0.147321
H	2.757112812	-3.703031512	5.768750174	0.169741
H	1.094212464	-3.538426185	5.201199982	0.1532
H	2.401215861	-2.737875537	4.323724999	0.135096
H	2.265995669	-7.502228741	6.231803364	0.004051
H	2.056385844	-5.809097515	6.62221127	0.011174
H	4.750204159	-7.152704954	5.979490302	0.082309
H	4.193146787	-6.663136378	7.592152846	0.072719
H	4.549581152	-5.439625995	6.358944066	0.085243
H	3.851004878	-8.820214609	3.231323275	0.092091
H	2.120838361	-9.113320906	3.057905955	0.066953
H	2.813952205	-8.831265239	4.658374307	0.136906
H	2.398151217	-6.843112327	-3.360506739	0.122289
H	1.480935881	-7.785591366	-2.186083398	0.086256
H	3.233328318	-7.653251949	-2.030974801	0.053744
H	2.245967436	-4.906506936	-4.097722211	-0.008122
H	2.062876431	-3.225374258	-3.645915545	-0.005382
H	-0.222781818	-5.283144227	-3.736507543	0.07638
H	0.067336675	-3.998334962	-4.926522477	0.068459
H	-0.411128058	-3.589179069	-3.269785253	0.086616
H	0.407439878	-2.046793582	-1.037473165	0.181376
H	1.874900914	-1.845847291	-1.996440187	0.185665
H	1.926766076	-1.645435924	-0.233792448	0.1579
H	0.588152916	-8.502365367	0.477556598	0.095181
H	0.954955385	-10.84632117	-0.184771088	0.157472
H	5.22624568	-10.18815869	-0.025339536	0.146542
H	4.822508519	-7.851376049	0.638963782	0.114593
H	1.406328878	-12.90412944	0.360200817	0.042214
H	1.637243969	-12.90958418	-2.165801121	0.011826
H	5.676711493	-12.04797351	-1.29026524	0.224062
H	5.333060751	-13.75517091	-0.929102085	0.224576
H	5.486493755	-12.58179993	0.400001328	0.197449
H	2.604222193	-14.03162417	-3.975682933	0.064304
H	3.504952768	-14.61999874	-2.592103725	0.01143
H	4.943119713	-14.13444073	-4.58469643	0.04107
H	5.448202315	-13.21020291	-3.188067775	-0.006986
H	3.802376403	-12.16449194	-5.543423467	0.051055
H	5.457624788	-11.7289219	-5.134108097	0.045532
H	3.835206034	-9.965475599	-4.380847678	0.026531
H	4.662803215	-10.67137375	-3.004239233	-0.018849

H	2.178814936	-10.48074609	-2.611771995	0.04247
H	1.887446954	-11.472167	-4.034159568	0.092197
H	1.962346354	-13.59446386	2.516171284	0.083307
H	3.124506462	-12.33017669	2.159718846	0.021136
H	4.134375333	-14.31342987	3.328028469	0.041377
H	4.904916306	-14.03703633	1.780373646	0.007565
H	2.761246171	-16.13897727	2.367596244	0.057211
H	4.461947117	-16.4428521	2.032249573	0.051042
H	3.042366739	-16.84182094	0.002338282	0.034142
H	4.193037482	-15.54666885	-0.276384896	0.000716
H	1.884594504	-14.92759069	-1.093667956	0.013517
H	1.244519396	-15.23798832	0.506649841	0.065434

7.2 Table of optimised atomic positions and CHELPG charges for structure **2**.

	X/Å	Y/Å	Z/Å	Charge/e
N	4.797384036	-22.42417543	-4.78305897	-0.243552
C	4.473641354	-21.31251335	-3.994150509	-0.402712
C	3.586111343	-20.34796472	-4.476381899	0.367391
C	2.986634275	-20.42828303	-5.738046363	-0.359062
N	3.264795052	-21.5146383	-6.576809474	-0.255528
B	4.262047894	-22.71424299	-6.25452116	1.021991
C	5.656771132	-23.18990918	-4.082914407	0.430976
C	5.911844296	-22.59732419	-2.809642157	-0.442611
C	5.17554507	-21.41862823	-2.74597302	0.377717
C	2.069709808	-19.55458533	-6.414325884	0.260125
C	1.809657527	-20.13934623	-7.65038141	-0.405428
C	2.567724856	-21.34545119	-7.717990176	0.440412
C	3.429241022	-24.13121978	-6.261738385	-0.710735
C	5.520457841	-22.61688167	-7.279893365	-0.672405
C	6.257293912	-24.45682669	-4.591798989	-0.57283
C	6.849228173	-23.15259772	-1.778488429	0.26361
C	8.300237636	-22.66919529	-1.966359397	-0.322322
C	5.156252511	-20.47732584	-1.57800284	-0.511414
C	1.477931654	-18.25910348	-5.939867959	-0.331928
C	0.944217545	-19.60808965	-8.755060191	0.23854
C	1.720405311	-18.7270179	-9.752900007	-0.30353
C	2.614796469	-22.28264512	-8.877658712	-0.58469
C	3.258565391	-19.17880204	-3.61779042	-0.069369
C	4.062090081	-18.03375627	-3.644884779	0.177812
C	3.735090811	-16.92103499	-2.876760073	-0.511193
C	2.593123495	-16.95074791	-2.062643896	0.342514
C	1.790426122	-18.10341094	-2.01748492	-0.33753
C	2.127915695	-19.20704576	-2.790142878	0.055679
P	2.090048655	-15.48049563	-1.178332302	0.048428
C	3.466111547	-14.33465218	-1.070328038	0.322134
C	0.714369039	-14.70904415	-2.034936981	0.332974
C	1.563832248	-15.92970627	0.495557035	-0.598577
C	4.571171769	-14.65739919	-0.26424892	-0.252262
C	5.654807128	-13.7872762	-0.201878646	-0.049507
C	5.643036159	-12.60114649	-0.940645643	-0.139095
C	4.549040169	-12.28272228	-1.745243585	0.002867
C	3.457361567	-13.14654837	-1.814598475	-0.354324
C	-0.011765575	-13.68037873	-1.410943932	-0.255563
C	-1.062866303	-13.07096229	-2.089075661	-0.066207
C	-1.390685015	-13.47988381	-3.384430567	-0.117292
C	-0.667671603	-14.49807513	-4.006080262	-0.003216
C	0.386401996	-15.11636095	-3.336341973	-0.356469

H	3.42035753	-24.60740415	-5.273781065	0.107854
H	3.838946652	-24.86708943	-6.965609688	0.119482
H	2.375899399	-23.98729495	-6.531049328	0.105483
H	6.02109377	-21.64133472	-7.217515293	0.094894
H	5.21687062	-22.75784025	-8.323296109	0.099002
H	6.278272254	-23.381937	-7.076829449	0.101158
H	6.33502968	-25.18716319	-3.780842108	0.175188
H	7.273153918	-24.27766633	-4.964730204	0.173053
H	5.672889192	-24.88937952	-5.401738154	0.156317
H	6.827844692	-24.24760202	-1.816368525	-0.004148
H	6.50318458	-22.87660271	-0.776021078	-0.014052
H	8.682783574	-22.95632157	-2.950289787	0.093572
H	8.957570083	-23.10062685	-1.205247702	0.074005
H	8.356501537	-21.57862398	-1.892759348	0.086037
H	4.140537793	-20.26569224	-1.229855002	0.110827
H	5.624395034	-19.51549918	-1.81700278	0.122129
H	5.71054377	-20.91252119	-0.742958173	0.168758
H	2.248357726	-17.5222238	-5.688383711	0.07394
H	0.848774965	-18.39086965	-5.052532711	0.073303
H	0.854268608	-17.82885995	-6.727321844	0.132858
H	0.113712106	-19.03167934	-8.332009631	-0.01089
H	0.487116874	-20.44475489	-9.295439304	-0.001527
H	2.156511519	-17.86060772	-9.245598231	0.077858
H	1.063123372	-18.36582001	-10.54988139	0.071163
H	2.538794658	-19.28800123	-10.21356175	0.089999
H	3.289595998	-21.90086159	-9.653528924	0.176258
H	1.621676115	-22.37159565	-9.328315982	0.175331
H	2.96091883	-23.27324803	-8.588743365	0.160479
H	4.940344291	-18.01690623	-4.28144204	0.084856
H	4.357637408	-16.03304442	-2.911179586	0.211907
H	0.899300304	-18.13662162	-1.398115988	0.170402
H	1.51079487	-20.09890231	-2.7659596	0.110965
H	0.748815096	-16.65445153	0.441605167	0.222587
H	1.21942849	-15.03634094	1.020747197	0.218445
H	2.405262962	-16.36718225	1.036974553	0.220632
H	4.591836649	-15.58354546	0.302386682	0.160218
H	6.507839057	-14.0336038	0.421339515	0.13889
H	6.490169328	-11.92520479	-0.887915808	0.151758
H	4.543046646	-11.3622416	-2.319166668	0.129913
H	2.604938571	-12.90117365	-2.439095803	0.18151
H	0.242944294	-13.35112802	-0.408045549	0.160134
H	-1.626333863	-12.27866652	-1.607909232	0.142736
H	-2.212710734	-13.00290968	-3.907988606	0.14643
H	-0.92373288	-14.81493582	-5.011510668	0.123173
H	0.946562566	-15.91140531	-3.816514304	0.183294

8 Crystallographic data for compound 3

Crystal structure data for ljh160010_3_fa was collected on a Xcalibur, Atlas, Gemini ultra-diffractometer equipped with an fine-focus sealed X-ray tube (λ CuKα = 1.54184 Å) and an Oxford Cryosystems CryostreamPlus open-flow N2 cooling device. Cell refinement, data collection and data reduction were undertaken via software CrysAlisPro 1.171.38.42b (Rigaku OD, 2015). Intensities were corrected for absorption using CrysAlisPro 1.171.38.42b (Rigaku Oxford Diffraction, 2015) Analytical numeric absorption correction using a multifaceted crystal model based on expressions derived by R.C. Clark & J.S. Reid.[9] Empirical absorption correction using spherical harmonics, implemented in SCALE3 ABSPACK scaling algorithm. The structure was solved using XT[10] and refined by XL[11] through the Olex2 interface.[12]

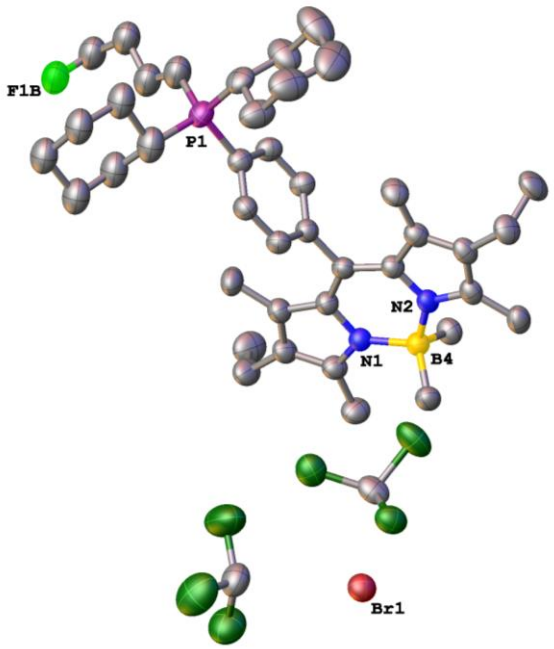


Figure S16. ORTEP plot with atoms of major component of **3** drawn as 50% probability ellipsoids.

There is both positional and occupational disorder manifest in the structure. One cyclohexyl group and a fluoroalkyl chain are disordered across two positions and in both positions the fluoralkyl chain is disordered over many different orientations, two of which have been incorporated into this model. In addition to this disorder one of the three chloroform molecules present in the structure has been modelled as disordered over two positions. The occupancies of the disordered parts have were allowed to refine with isotropic atom treatment. Upon convergence, the occupancies were fixed before anisotropy was added to the model. The ADPs of the disordered atoms were constrained using the EADP card where appropriate. The geometries of the disordered parts were restrained using either the SADI or the SAME card.

Identification code	ljh160010_3_fa
Empirical formula	$C_{44}H_{65}BBrCl_9FN_2P$
Formula weight	1081.72
Temperature/K	150.0(2)
Crystal system	monoclinic
Space group	$P2_1/c$
a/Å	24.7264(7)
b/Å	9.6603(2)
c/Å	23.4725(7)
α/°	90
β/°	109.127(3)
γ/°	90
Volume/Å^3	5297.2(3)
Z	4
ρ_{calc}g/cm^3	1.356
μ/mm^{-1}	5.794
F(000)	2240.0
Crystal size/mm^3	0.47 × 0.06 × 0.02
Radiation	CuKα (λ = 1.54184)
2Θ range for data collection/°	7.568 to 133.966
Index ranges	-29 ≤ h ≤ 29, -9 ≤ k ≤ 11, -27 ≤ l ≤ 27
Reflections collected	38266
Independent reflections	9392 [R_{int} = 0.0860, R_{sigma} = 0.0607]
Data/restraints/parameters	9392/41/549
Goodness-of-fit on F^2	1.020
Final R indexes [I>=2σ (I)]	R_1 = 0.0685, wR_2 = 0.1786
Final R indexes [all data]	R_1 = 0.1093, wR_2 = 0.2101

Table of Bond Lengths for 3 (ljh160010_3_fa.)

Atom	Atom	Length/Å	Atom	Atom	Length/Å
Cl1	C51	1.747(7)	C26	C27	1.517(9)
Cl2	C51	1.755(6)	C27	C28	1.510(14)
Cl3	C51	1.756(6)	C28	C29	1.535(14)
Cl4	C52	1.747(7)	C29	C30	1.513(10)
Cl5	C52	1.716(8)	C31A	C32A	1.441(16)
Cl6	C52	1.748(8)	C31A	C36A	1.714(18)
P1	C22	1.795(5)	C31A	C37A	1.44(2)
P1	C25	1.821(6)	C31A	C37B	1.24(3)
P1	C31A	1.800(8)	C31B	C43	1.438(16)
P1	C31B	1.805(7)	C31B	C37D	1.451(17)
N1	C3	1.335(6)	C31B	C32B	1.433(16)
N1	C9	1.403(6)	C31B	C36B	1.653(17)
N1	B4	1.588(7)	C49	B4	1.616(8)
N2	C5	1.343(6)	C50	B4	1.622(8)
N2	C10	1.391(6)	Cl7B	C53B	1.721(10)
N2	B4	1.592(6)	Cl8B	C53B	1.766(11)
C1	C2	1.385(7)	Cl9B	C53B	1.74(3)
C1	C9	1.424(7)	C43	C38C	1.477(18)
C1	C11	1.503(8)	C38C	C39C	1.474(18)
C2	C3	1.405(7)	C39C	F1C	1.316(19)
C2	C12	1.502(7)	C32A	C33A	1.49(2)
C3	C14	1.496(7)	C33A	C34A	1.49(2)
C5	C6	1.413(7)	C34A	C35A	1.55(2)
C5	C15	1.484(7)	C35A	C36A	1.58(2)
C6	C7	1.376(7)	Cl7A	C53A	1.716(15)
C6	C16	1.504(7)	Cl8A	C53A	1.736(16)
C7	C10	1.431(6)	Cl9A	C53A	1.74(4)
C7	C18	1.493(8)	C37A	C38A	1.461(19)
C8	C9	1.392(6)	C38A	C39A	1.44(2)
C8	C10	1.400(7)	C39A	F1A	1.30(2)
C8	C19	1.488(7)	C37D	C38D	1.477(18)
C12	C13	1.509(10)	C38D	C39D	1.454(18)
C16	C17	1.518(9)	C39D	F1D	1.330(19)
C19	C20	1.385(7)	C37B	C38B	1.46(2)
C19	C24	1.387(7)	C38B	C39B	1.45(2)
C20	C21	1.382(7)	C39B	F1B	1.318(19)
C21	C22	1.400(8)	C32B	C33B	1.51(2)
C22	C23	1.401(7)	C33B	C34B	1.59(2)
C23	C24	1.386(7)	C34B	C35B	1.48(2)
C25	C26	1.521(9)	C35B	C36B	1.57(2)
C25	C30	1.535(10)			

Table of Bond Angles for 3 (ljh160010_3_fa.)

Atom	Atom	Atom	Angle/˚	Atom	Atom	Atom	Angle/˚
C22	P1	C25	107.1(2)	C28	C27	C26	110.0(7)
C22	P1	C31A	109.6(3)	C27	C28	C29	111.5(7)
C22	P1	C31B	109.2(3)	C30	C29	C28	110.5(7)
C31A	P1	C25	110.6(3)	C29	C30	C25	109.8(6)
C31A	P1	C31B	110.5(3)	C32A	C31A	P1	117.5(8)
C31B	P1	C25	109.7(3)	C32A	C31A	C36A	106.6(9)
C3	N1	C9	107.3(4)	C36A	C31A	P1	106.9(7)
C3	N1	B4	126.7(4)	C37A	C31A	P1	118.8(13)
C9	N1	B4	125.8(4)	C37B	C31A	P1	130.7(15)
C5	N2	C10	107.5(4)	C43	C31B	P1	132.3(13)
C5	N2	B4	126.6(4)	C37D	C31B	P1	124.4(13)
C10	N2	B4	125.8(4)	C32B	C31B	P1	116.8(8)
C2	C1	C9	106.4(4)	C32B	C31B	C36B	107.3(9)
C2	C1	C11	125.1(4)	C36B	C31B	P1	106.7(7)
C9	C1	C11	128.5(4)	Cl1	C51	Cl2	110.8(3)
C1	C2	C3	107.3(4)	Cl1	C51	Cl3	111.4(3)
C1	C2	C12	127.6(5)	Cl2	C51	Cl3	109.5(3)
C3	C2	C12	125.0(5)	Cl4	C52	Cl6	108.6(4)
N1	C3	C2	110.7(4)	Cl5	C52	Cl4	111.8(4)
N1	C3	C14	125.1(5)	Cl5	C52	Cl6	112.6(4)
C2	C3	C14	124.2(4)	N1	B4	N2	105.0(4)
N2	C5	C6	109.9(4)	N1	B4	C49	108.2(4)
N2	C5	C15	125.2(4)	N1	B4	C50	108.5(4)
C6	C5	C15	124.8(4)	N2	B4	C49	109.0(4)
C5	C6	C16	123.8(5)	N2	B4	C50	109.6(4)
C7	C6	C5	107.8(4)	C49	B4	C50	116.0(4)
C7	C6	C16	128.3(5)	C31B	C43	C38C	114(2)
C6	C7	C10	106.1(4)	C39C	C38C	C43	111.1(19)
C6	C7	C18	124.6(4)	F1C	C39C	C38C	119(2)
C10	C7	C18	129.3(5)	C31A	C32A	C33A	114.1(12)
C9	C8	C10	122.1(4)	C34A	C33A	C32A	108.4(13)
C9	C8	C19	118.9(4)	C33A	C34A	C35A	107.9(12)
C10	C8	C19	119.0(4)	C34A	C35A	C36A	109.7(14)
N1	C9	C1	108.2(4)	C35A	C36A	C31A	107.3(12)
C8	C9	N1	120.4(4)	Cl7B	C53B	Cl8B	111.6(6)
C8	C9	C1	131.4(5)	Cl7B	C53B	Cl9B	112.9(10)
N2	C10	C7	108.6(4)	Cl9B	C53B	Cl8B	109.9(17)
N2	C10	C8	120.7(4)	C31A	C37A	C38A	121(2)
C8	C10	C7	130.7(4)	C39A	C38A	C37A	114(2)
C2	C12	C13	111.9(5)	F1A	C39A	C38A	108.2(19)
C6	C16	C17	112.9(5)	C31B	C37D	C38D	115(2)
C20	C19	C8	120.5(4)	C39D	C38D	C37D	112.2(19)
C20	C19	C24	119.8(4)	F1D	C39D	C38D	121(2)
C24	C19	C8	119.6(4)	Cl7A	C53A	Cl8A	112.7(11)

C21	C20	C19	120.1(5)	Cl7A	C53A	Cl9A	113.3(18)
C20	C21	C22	120.3(5)	Cl9A	C53A	Cl8A	112(2)
C21	C22	P1	120.7(4)	C31A	C37B	C38B	121(3)
C21	C22	C23	119.6(5)	C39B	C38B	C37B	114(2)
C23	C22	P1	119.4(4)	F1B	C39B	C38B	107.1(19)
C24	C23	C22	119.1(5)	C31B	C32B	C33B	110.8(12)
C23	C24	C19	121.0(5)	C32B	C33B	C34B	109.4(13)
C26	C25	P1	111.5(4)	C35B	C34B	C33B	107.6(14)
C26	C25	C30	112.2(6)	C34B	C35B	C36B	112.1(13)
C30	C25	P1	110.8(4)	C35B	C36B	C31B	108.2(12)
C27	C26	C25	110.4(6)				

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